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MORPHOLOGICAL AND CHEMICAL STUDIES OF THE BLOOD OF CATTLE IN HEALTH AND DURING ANAPLASMOSIS[1]

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INTRODUCTION

The purpose of the present investigation was to obtain precise data on the total red-cell and white-cell counts, the differential white-cell counts, and the chemical constituents of the blood of cattle during anaplasmosis and in health, and thereby if possible to discover some factors in the behavior of the causative agent that would suggest a method of controlling the disease. The morphological readings were taken by the senior author and the chemical readings by the junior author. Each worked independently, the former at Beltsville, Md., and the latter at Washington, D. C. However, since all the blood samples were drawn at Beltsville under the direct supervision of the senior author, and since the same animals were used in the two investigations, the data are presented in a joint paper. The studies are being continued by the Zoological Division.

REVIEW OF LITERATURE

Smith and Kilborne (*15*)[2] studied the blood of cattle having anaplasmosis, although they believed the disease to be a mild form or a relapse of piroplasmosis characterized by secondary anemia resulting from the destruction of red blood cells. Theiler (*17*) demonstrated that these investigators were dealing with a specific disease entirely distinct from piroplasmosis, and proposed the name "anaplasmosis." He confirmed their data on the blood picture, and numerous other authors have likewise confirmed them.

Data on the morphology of the white blood cells were reported by De Kock and Quinlan (*4*). They studied the reticulo-endothelial system of cattle and sheep having anaplasmosis. In both non-splenectomized and splenectomized cases a monocytosis was characteristic of the clinical reaction and the period of convalescence. Frequently the observed monocytes, some of which were probably reticulo-endothelial cells, contained phagocytized erythrocytes in various stages of digestion. The authors believed that one of the functions of the reticulo-endothelial cells is thus to destroy infected erythrocytes. Confirmatory evidence for this belief was furnished

[1] Received for publication Mar. 24, 1936; issued November 1936.

[2] Reference is made by number (italic) to Literature Cited, p. 491.

by the removal of the spleen of carriers, for after this part of the reticulo-endothelial system was taken out a relapse occurred. The work of De Kock and Quinlan was confirmed by Rees (*11*, *12*).

A thoroughgoing statistical study of the white blood cells of cattle in health and during anaplasmosis was conducted by Sergent and his collaborators (*14*). In order to obtain the normal blood picture, they made 67 counts from 37 healthy cattle between the ages of 18 months and 2 years. The mean total white-cell count per cubic millimeter was 10,056. The mean percentages of the various types of cells, as shown by the differential count, were as follows: Lymphocytes, 59.50; monocytes, 7.50; neutrophiles, 22.00; eosinophiles, 10.00; and basophiles, 1.00. Eight cases of anaplasmosis were studied, in seven of which there was a mild leucocytosis, the increase above the average normal figure varying from 1,000 to 10,000 cells. In the differential count during the incubation, clinical, and convalescent periods there was little change from the normal. The average percentages for the clinical period were as follows: Lymphocytes, 62.85; monocytes, 6.80; neutrophiles, 23.70, with no shift to either the right or the left; eosinophiles, 5.70; and basophiles, 0.95. These figures show a slight increase of the lymphocytes and neutrophiles and a decrease of the eosinophiles, but the deviations were not beyond the normal range. In agreement with this work Livotov (*10*) found a leucocytosis during the clinical period, but in disagreement he reported a marked shift to the left of the neutrophiles and also an aneosinophilia. Stephan and his collaborators (*16*) studied 12 clinical cases and found the average percentages to be as follows: Lymphocytes, 47.17; monocytes, 27; neutrophiles, 25.50; eosinophiles, 0.33; and basophiles, 0. Judging from average normal readings, the data of Stephan et al. for anaplasmosis appear to show a relative decrease of lymphocytes, an increase of monocytes, and a marked decrease of eosinophiles; in 9 of their 12 cases there was an aneosinophilia. To summarize the preceding data on the morphological blood picture: There is general agreement that a leucocytosis occurs during anaplasmosis, but on the differential count there is some disagreement. The data of Sergent and his collaborators, for example, show no significant variations from the normal; those of Stephan and his collaborators show a relative lymphopenia, a marked monocytosis, a shift of the neutrophiles to the left, and an eosinopenia.

On the blood chemistry of cattle either in health or during anaplasmosis little work has been published. The concentrations of the constituents vary considerably in the two sexes, as is well shown by the studies of Amadon (*1*) on so-called milk fever. No complete range of data, covering a relatively large number of animals of different breeds and ages on a fixed diet and extending over a period of time sufficient to show seasonal variations, effect of pregnancy, and the like, exists. Moreover, with the use of various methods, different results have been obtained by different workers, and comparison of results is difficult. According to Braga (*3*), the urea of the blood serum rose above normal during the clinical attack of anaplasmosis and fell below normal during convalescence. Ducloux and Cordier (*5*, *v. 191*), in abstracts of their researches on the blood chemistry of cattle having anaplasmosis, published the following data on the sugar, urea, and bilirubin. During the crisis of the disease the blood sugar rose above the normal level of about 601 mg to 1,000 mg per liter.

During the late clinical period of a severe case the urea rose from the normal level of about 263 mg to 2,315 mg per liter. By both the direct and the indirect Van den Bergh reactions, progressive increase of serum bilirubin was noted during the clinical period and a decrease during convalescence. Ducloux and Cordier used Fehling solution for the quantitative analysis of the sugar after precipitating the proteins with Patein's reagent. For the urea determination they used a hypobromite method and Yvon's ureometer.

EXPERIMENTAL PROCEDURE AND APPARATUS

In the present work, five healthy bulls between the ages of 1½ and 2 years were used. Experimental control was facilitated (1) by keeping the bulls in a barn which was free from ticks and flies, thereby preventing transmission of infection by such means; (2) by feeding uniform rations; and (3) by drawing the blood always at 8 a. m., before the morning feeding. For each 500 pounds of body weight the daily ration was 2½ pounds of equal parts of oats and wheat bran and 12½ pounds of alfalfa hay. However, during the period of acute clinical symptoms the cattle stopped eating entirely. To evaluate uncontrollable factors producing variability in the constituents of the blood, the following procedure was followed: All five bulls were maintained uninfected for 6 weeks during a preliminary period of testing, from May 23 to July 6, 1934, and one of them, bull 124, was held uninfected throughout the 3 months' time required for the joint morphological and chemical testing and for several months beyond this period. The susceptibility of this bull was then established by injecting virulent blood; a fatal case of anaplasmosis resulted. On July 6 four of the bulls, nos. 120, 122, 123, and 125, were injected intravenously with virulent blood.

For the morphological readings the blood was collected from the ear by lancet puncture directly into the diluting pipettes, the same pipettes and other equipment being used for all the readings, which were taken personally by the senior writer. For the differential count of the leucocytes glass slides were used; Schilling's (*13*) formula for the neutrophiles was followed. For each chemical analysis two samples of blood were drawn from the jugular vein. The first one of 15 cc was drawn into a test tube, where it was allowed to clot. From this sample the phosphorus, serum proteins, and calcium were determined. From the second sample of 20 cc the oxygen and carbon dioxide content, oxygen capacity, hemoglobin, sugar, and urea were determined; the blood was drawn anaerobically and clotting was prevented as follows: The shank of a glass syringe was provided with 4 mg of dry anticoagulant for each cubic centimeter of blood; the anticoagulant consisted of neutral potassium oxalate, 3 parts, and ammonium fluoride, 1 part (for urea determinations potassium fluoride was substituted for ammonium fluoride). The air was exhausted from the syringe by the plunger, which was oiled with liquid petrolatum, and an airtight cap was fitted on the nipple. While the blood was flowing freely from the bleeding needle, the nipple was uncapped and attached and the syringe filled by venous blood pressure. The syringe was then capped, rotated to mix the anticoagulant, and transported to the laboratory in a box especially designed to carry it upright so that the weight of the plunger was always on the blood. The analyses

were begun within 30 to 40 minutes, and except for the calcium analysis, which required an overnight standing period, were completed on the same day that the blood was drawn.

The filtrate for determination of sugar and urea was prepared by Haden's (*8*) modification of Folin-Wu precipitation, using 8 volumes of $\frac{N}{12}$ sulphuric acid and 1 volume of 10-percent sodium tungstate solution to 1 volume of blood. The filtrate for calcium and phosphorus determinations was prepared by adding 12 volumes of 10-percent trichloracetic acid to 3 volumes of serum. Sugar was determined by the colorimetric method of Benedict (*2*); phosphorus by that of Fiske and Subbarow (*6*); serum proteins by Greenberg's method (*7*), the proteins not being separated; calcium by the manometric method of Van Slyke and Sendroy (*19*); urea by the method of Leiboff and Kahn (*9*); carbon dioxide, oxygen content, oxygen capacity, and hemoglobin by the method of Van Slyke and Neill (*18*). Carbon dioxide in the serum and that in the hemoglobin were calculated from carbon dioxide and oxygen capacity, respectively, in the whole blood.

Because of the laborious procedure involved in determining urea, no determinations were made for the normal period (May 23 to July 2) of any of the bulls. However, bull 124 was normal throughout the entire testing period, and since urea determinations were obtained on this animal after July 2 it was possible by means of these to determine the effect of the disease on the urea.

EXPERIMENTAL DATA

MORPHOLOGICAL CONSTITUENTS

The morphological data (table 1) obtained during the normal and the incubation periods are in agreement with those of previous investigators. Therefore, only the averages for these periods and only the averages for the control bull 124 are reported. The data of table 1 show that the weights of the cattle were adversely affected by anaplasmosis. During the clinical and subclinical periods, despite the fact that the bulls were young, rapidly growing animals, they actually lost weight.

TABLE 1.—*Morphological constituents of the blood of five bulls in health and of four of them during subsequent anaplasmosis*

Bull no.	Period	Date [1]	Weight	Anaplasmata per 1,000 red cells	Red-cell count per cubic millimeter	White-cell count per cubic millimeter	Differential white-cell count							
							Lymphocytes	Monocytes	Neutrophiles			Eosinophiles	Basophiles	Imperfectly stained or smudged
									Juveniles	Band forms	Segmented			
		1934	*Pounds*	*Number*	*Millions*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
120	Normal	May 23–July 6	610	0	7.24	11,943	62.5	6.0	0	2.1	13.9	12.9	1.5	1.1
	Incubation	July 6–20	618	0	6.12	11,290	61.9	10.7	0	2.7	11.7	10.5	1.3	1.2
	Clinical	July 23		118	3.88	9,800	61.0	22.0	0	5.0	8.5	2.0	.5	1.0
		July 25	593	120	2.62	11,600	68.0	19.5	0	3.0	4.5	2.5	.5	2.0
		July 27		93	1.79	11,600	65.5	10.0	.5	9.5	6.5	5.0	0	3.0
		July 30	594	78	2.01	13,560	47.0	10.0	5.5	21.0	13.5	2.5	0	.5
	Average		593	102	2.57	11,640	60.4	15.4	1.5	9.6	8.2	3.0	.3	1.6
	Subclinical	Aug. 3		5	3.36	10,560	59.0	17.5	1.5	8.0	10.0	2.5	.5	1.0
		Aug. 6	614	0	3.65	12,160	62.0	16.0	1.0	10.0	6.0	4.0	0	1.0
		Aug. 10		0	3.10	11,040	69.5	10.5	0	2.5	8.5	4.5	.5	4.0
		Aug. 17		0	3.30	8,960	78.5	12.0	1.0	0	0	7.0	.5	1.0
		Aug. 22	587	0	3.48	19,320	70.0	12.0	0	10.5	4.5	3.0	0	0
	Average		600	1	3.38	12,408	67.8	13.6	.7	6.2	5.8	4.2	.3	1.4
122	Normal	May 23–July 6	302	0	6.29	11,700	81.4	5.9	0	1.1	6.9	2.9	.8	1.0
	Incubation	July 6–20	326	0	5.83	11,340	76.7	9.2	0	1.0	5.5	6.6	.25	.75
	Clinical	July 23	329	112	3.64	7,400	62.5	22.0	0	8.0	4.5	.5	0	2.5
		July 25		225	1.76	11,600	69.5	10.5	5.5	7.5	6.0	0	0	1.0
		July 27		143	1.31	11,760	45.0	24.5	8.0	12.5	9.5	.5	0	0
		July 30		100	.76	35,040	44.0	29.0	4.0	15.0	7.0	1.0	0	0
	Average		329	145	1.87	16,450	55.2	21.5	4.4	10.7	6.7	.5	0	.9
	Subclinical	Aug. 3	280	4	1.34	23,440	64.5	8.0	2.5	10.0	14.5	0	0	.5
		Aug. 6		0	1.62	8,640	61.0	8.0	3.0	15.0	10.0	0	0	3.0
		Aug. 10		0	2.15	12,960	82.5	5.5	0	5.5	6.0	.5	0	0
		Aug. 17		0	3.76	13,080	80.5	15.0	0	0	2.0	2.0	0	.5
		Aug. 22	303	0	4.50	20,440	64.5	8.5	0	15.0	9.0	3.0	0	0
	Average		291	1	2.67	15,712	70.6	9.0	1.1	9.1	8.3	1.1	0	.8

[1] On July 6 bulls 120, 122, 123, and 125 were injected with virulent blood. Bull 120 reacted to anaplasmosis on July 21; bulls 122 and 125 on July 23, and bull 123 on July 20.

TABLE 1.—*Morphological constituents of the blood of five bulls in health and of four of them during subsequent anaplasmosis*—Continued

Bull no.	Period	Date	Weight	Anaplasmata per 1,000 red cells	Red-cell count per cubic millimeter	White-cell count per cubic millimeter	Differential white-cell count							
							Lymphocytes	Monocytes	Neutrophiles			Eosinophiles	Basophiles	Imperfectly stained or smudged
									Juveniles	Band forms	Segmented			
		1934	*Pounds*	*Number*	*Millions*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
123	Normal	May 23–July 6	450	0	7.15	8,069	60.6	6.9	0	3.5	24.3	2.9	.9	.9
	Incubation	July 6–20	482	0	5.90	5,760	60.9	9.7	0	5.4	20.1	2.1	.5	1.3
	Clinical	July 23	478	175	2.88	5,920	66.0	20.0	0	5.0	9.0	0	0	0
		July 25		0	1.90	6,760	68.0	23.0	0	3.0	2.0	2.0	0	2.0
		July 27	457	148	2.52	9,680	57.0	24.5	2.0	6.0	6.0	1.0	.5	3.0
		July 30		220	1.66	8,520	54.0	26.0	1.0	14.0	3.0	0	0	2.0
	Average		467	136	2.24	7,720	61.2	23.4	.8	7.0	5.0	.8	0	1.8
	Subclinical	Aug. 3		3	2.58	8,400	57.5	22.0	1.0	14.5	3.5	0	1.5	0
		Aug. 6	465	0	2.86	8,640	53.0	17.0	0	15.0	12.0	0	0	3.0
		Aug. 10		0	3.40	7,480	56.5	9.0	0	10.0	20.0	.5	0	4.0
		Aug. 17	497	0	3.60	8,880	73.0	15.0	.5	1.0	8.0	1.5	1.0	0
		Aug. 23	494	0	3.10	8,800	63.0	14.5	0	4.5	16.5	.5	.5	.5
	Average		485	.6	3.11	8,440	60.6	15.5	.3	9.0	12.0	.5	.6	1.5
125	Normal	May 23–July 6	568	0	7.62	10,191	60.1	7.1	0	2.5	25.1	3.4	.8	1.0
	Incubation	July 6–20	612	0	6.62	8,030	65.6	12.0	0	2.9	15.6	2.4	.4	1.1
	Clinical	July 23	568	26	4.16	9,600	59.0	9.5	0	2.5	25.0	3.0	1.0	0
		July 25		35	4.96	11,320	63.0	23.0	0	1.0	4.5	7.0	1.5	0
		July 27		54	4.40	11,600	56.0	22.0	.5	5.5	12.0	1.5	0	2.5
		July 30		80	2.44	12,880	46.0	22.0	0	16.0	11.0	1.0	0	4.0
		Aug. 3		25	1.19	16,900	48.0	25.0	2.0	16.0	8.0	0	0	1.0
		Aug. 6 [2]		10	1.28	14,560	55.0	25.0	3.0	10.0	5.0	0	0	2.0
	Average		568	38	3.07	12,810	54.5	21.1	.9	8.5	10.9	2.1	.4	1.6
124 (control)	Normal	May 23–July 6	596	0	6.44	11,517	66.9	5.6	0	0	17.6	7.5	1.0	1.4
	Normal control of incubation	July 10–20	637	0	6.11	8,050	67.0	9.4	0	1.5	14.1	6.8	.5	.7
	Normal control of clinical	July 23–30	654	0	6.11	7,760	61.9	10.5	0	1.0	15.0	7.7	1.4	2.5
	Normal control of subclinical	Aug. 3–22	696	0	6.50	9,480	56.5	9.2	0	3.7	18.5	10.4	.7	1.0
Average [3]	Normal		482	0	7.07	10,476	66.2	6.5	0	2.3	17.6	5.6	1.0	1.0
	Incubation		509	0	6.12	9,105	66.3	10.4	0	3.0	13.2	5.4	.6	1.1
	Clinical		489	105	2.44	12,155	57.8	20.4	1.9	8.9	7.7	1.6	.2	1.5
	Subclinical [3]		459	1	3.19	12,187	66.3	12.7	.7	8.1	8.7	1.9	.3	1.2

[2] Bull 125 was killed on this date to obtain material for pathological study as it appeared that he would not live through the night.
[3] Averages of bulls 120, 122, and 123.

The temperatures deviated from normal during clinical anaplasmosis only, and the graphs for all the affected animals were very much alike; therefore, the temperature chart of bull 122 only is presented (fig. 1). As figure 1 shows, the onset of the clinical period was marked by an abrupt rise in temperature and the termination of this period by an equally abrupt drop. Comparison with table 1 shows a correspondingly abrupt appearance, increase, and marked decrease of anaplasmata. However, a lack of correlation between the severity of attack and the concentration of anaplasmata in the red cells is strikingly shown by the data for bull 125, as compared with those for the other three bulls, for, although the reaction in bull 125 was severe, the counts for anaplasmata were low. On August 6 this animal was killed to obtain material for pathological study since it appeared that he would not live through the night.

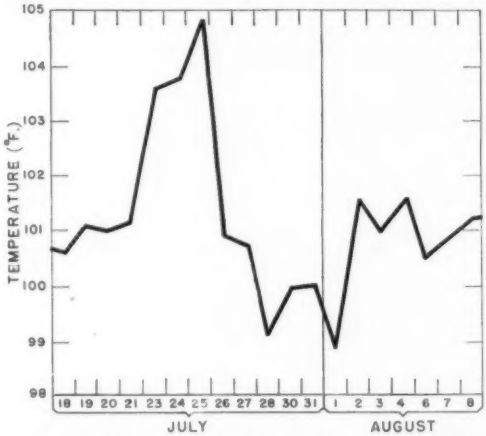

FIGURE 1.—Temperature chart of bull 122 from July 18 to August 8, 1934. A positive diagnosis of anaplasmosis was made on July 23 and the clinical period extended from that date to July 30.

In all cases during the clinical period the drop in the red-cell count was very great; in the case of bull 122 it fell below 1 million. It is remarkable that this bull recovered.

In agreement with the data of previous investigators, those of the present paper show a marked leucocytosis during the clinical and the subclinical periods. In showing a marked differential monocytosis, a relative lymphopenia during the clinical period, a shift of the neutrophiles to the left, and a marked eosinopenia, the data of the present writers appear to be in general agreement with those of most of the investigators previously cited, especially with those of Stephan and his coworkers (*16*), but in disagreement with those of Sergent and his coworkers (*14*).

CHEMICAL CONSTITUENTS

As pointed out previously, there are no chemical data in the literature with which to compare many of the data of the present paper. For this reason all the chemical data obtained, and the averages as well, are reported in table 2.

TABLE 2.—*Chemical constituents of the blood of five bulls in health and of four of them during subsequent anaplasmosis*

Bull no.	Period	Date	Quantity of constituent per 100 cc— In whole blood: Sugar	Phosphorus	Serum proteins	Hemoglobin	Urea	In serum, calcium	Volume of constituent— In whole blood, carbon dioxide	In serum, carbon dioxide	In whole blood: Oxygen content	Oxygen capacity	Results of Van den Bergh direct test for serum bilirubin
		1934	*Mgs*	*Mgs*	*Mgs*	*Gms*	*Mgs*	*Mgs*	*Percent*	*Percent*	*Percent*	*Percent*	
	Normal	May 23	65.50	7.62	8.66	11.26	.....	12.39	57.58	66.50	6.02	15.59	
		May 29	72.72	8.00	9.81	12.41	.....	11.97	59.26	29.93	9.48	17.14	
		June 4	60.98	8.00	9.45	11.67	.....	14.22	60.57	70.26	5.90	16.14	
		June 12	70.42	7.77	8.00	10.92	.....	11.20	68.82	72.31	8.52	15.14	
		June 20	56.34	6.20	9.63	12.53	.....	10.95	69.83	71.78	6.17	17.31	
		June 26	55.55	6.90	7.65	11.91	.....	11.44	57.26	66.92	8.78	16.46	
		July 2	77.52	8.26	9.12	10.85	.....	12.20	59.13	68.00	6.70	15.04	
	Average		65.62	7.54	8.90	11.68	.....	12.05	60.49	63.68	7.37	16.12	
	Incubation	July 10	69.58	6.66	8.66	9.58	23.53	11.89	62.12	70.20	7.17	13.34	Negative.
		July 13	50.00	6.78	8.39	11.82	17.54	11.93	59.53	69.35	8.28	16.34	Do.
		July 17	64.95	6.06	7.88	11.04	12.12	13.56	56.78	65.58	8.13	15.30	Do.
		July 20	59.52	5.26	8.39	9.62	14.39	12.78	60.52	68.51	5.88	13.39	Do.
	Average		61.01	6.19	8.33	10.51	16.89	12.54	59.74	68.41	7.36	14.59	
120	Clinical	July 23	64.10	3.64	7.22	6.96	15.15	12.98	55.33	60.31	3.59	9.70	Delayed, faint.
		July 25	83.68	4.94	9.29	2.79	14.51	11.07	58.01	60.33	1.66	4.24	Delayed, medium
		July 27	65.57	6.92	7.65	3.66	11.90	10.15	62.29	65.40	.71	5.42	Do
		July 30	50 00	6.25	6.34	3.79	10.90	11.14	63.83	67.66	1.18	6.37	Delayed, faint.
		Aug. 1	62.11	6.66	7.88	6.14	10.70	10.96	63.67	68.95	1.43	8.73	Do.
	Average		65.09	5.68	7.68	4.67	12.63	11.26	60.63	64.53	1.71	6.89	
	Subclinical	Aug. 3	64.10	6.66	7.54	6.88	12.50	15.67	62.86	68.52	2.61	9 73	Negative.
		Aug. 6	55.87	5.84	7.76	7.53	11.46	17.49	61.31	67.44	2.67	10.59	Do.
		Aug. 10	57.16	6.66	7.88	7.53	12.50	14.24	63.67	69.71	4.27	10.54	Do.
		Aug. 17	62.89	6.40	8.00	6.30	15.38	10.04	63.81	69.19	4.81	8.90	Do.
		Aug. 22	63.30	6.66	9.00	10.92	16.66	11.20	62.82	72.24	8.52	15.14	Do.
	Average		60.66	6.44	8.04	7.83	13.70	13.73	62.89	69.42	4.58	10.98	

122	Normal	May 23	75.40	8.88	8.32	10.68		10.90	67.96	78.15	7.70	14.82	
		May 29	81.63	7.27	7.22	10.55		11.70	64.27	73.59	7.54	14.78	
		June 4	74.07	9.76	9.45	10.25		11.69	59.95	67.83	5.90	14.24	
		June 12	79.37	7.14	10.61	8.98		10.96	62.31	69.91	6.62	12.54	
		June 20	62.38	8.25	7.54	11.26		10.23	56.51	65.44	8.81	15.60	
		June 26	58.48	6.25	6.50	11.44		11.38	62.86	72.92	8.55	15.83	
		July 2	74.07	6.15	6.27	9.41		11.00	57.75	65.26	8.02	13.12	
	Average		72.20	7.67	7.99	10.37		11.12	61.66	70.44	7.59	14.42	
	Incubation	July 10	60.97	6.84	7.22	7.86	20.00	11.39	61.09	67.48	6.45	11.04	
		July 13	57.14	6.72	6.93	10.04	23.53	11.20	57.50	65.55	9.24	13.96	Negative.
		July 17	64.31	6.45	6.12	9.79	18.18	12.59	51.88	58.83	8.42	13.62	Do.
		July 20	64.31	5.63	7.03	9.44	19.61	12.49	59.01	66.68	7.76	13.16	Do.
	Average		61.68	6.41	6.82	9.28	20.33	11.92	57.37	64.63	7.97	12.94	
	Clinical	July 23	62.92	5.71	6.84	6.15	20.83	11.76	61.40	66.31	1.91	8.75	Do.
		July 25	75.19	4.79	6.50	3.31	20.20	9.39	56.23	58.76	.47	4.95	Delayed, strong.
		July 27	44.44	5.13	7.22	1.92	16.20	9.92	46.35	47.74	.24	3.07	Delayed, medium.
		July 30	31.25	6.06	6.27	1.56	25.32	10.65	30.40	31.22	.00	2.59	Diphasic, strong.
		Aug. 1	77.82	7.27	8.66	2.28	30.77	9.98	42.70	44.19	.00	3.56	Prompt, strong.
	Average		58.32	5.79	7.10	3.04	22.66	10.34	47.42	49.64	.52	4.58	
	Subclinical	Aug. 3	70.67	9.64	6.66	3.00	12.50	11.27	52.42	54.68	.00	4.51	Diphasic, strong.
		Aug. 6	53.62	6.66	7.43	5.01	13.33	16.90	56.85	60.83	1.21	7.22	Delayed, medium.
		Aug. 10	64.93	5.40	6.94	8.94	15.15	15.34	44.28	47.37	2.85	7.12	Negative.
		Aug. 17	75.18	5.00	6.75	6.80	15.38	11.00	65.63	69.57	6.98	9.62	Do.
		Aug. 22	75.47	4.21	7.42	8.98	16.66	12.20	62.31	69.79	6.62	12.54	Do.
	Average		67.97	6.18	7.04	6.56	14.60	13.34	56.30	60.45	3.53	8.20	

[1] On July 6 bulls 120, 122, 123, and 125 were injected with virulent blood. Bull 120 reacted to anaplasmosis on July 21, bulls 122 and 125 on July 23, and bull 123 on July 20.

TABLE 2.—*Chemical constituents of the blood of five bulls in health and of four of them during subsequent anaplasmosis*—Continued

Bull no.	Period	Date	Quantity of constituent per 100 cc— In whole blood: Sugar	Phosphorus	Serum proteins	Hemoglobin	Urea	In serum, calcium	Volume of constituent— In whole blood, carbon dioxide	In serum, carbon dioxide	In whole blood: Oxygen content	Oxygen capacity	Results of Van den Bergh direct test for serum bilirubin
		1934	*Mgs*	*Mgs*	*Mgs*	*Gms*	*Mgs*	*Mgs*	*Percent*	*Percent*	*Percent*	*Percent*	
123	Normal	May 23	72.70	8.18	7.12	10.86	.....	11.15	66.92	77.16	5.05	15.06	
		May 29	82.47	7.62	8.81	11.01	.....	10.97	43.23	49.93	8.00	15.26	
		June 4	69.32	7.08	7.22	8.27	.....	11.20	61.08	67.49	4.98	11.58	
		June 12	72.46	6.06	6.93	11.06	.....	10.23	62.31	72.28	5.44	15.34	
		June 20	54.05	7.92	7.03	11.06	.....	10.23	58.04	66.92	5.24	15.34	
		June 26	61.35	6.15	6.66	10.26	.....	11.98	53.34	61.02	6.29	14.25	
		July 2	78.12	6.80	6.84	8.69	.....	11.97	57.75	64.66	6.87	12.18	
	Average		70.07	7.12	7.23	10.17	.....	11.10	57.52	65.64	5.98	14.14	
	Incubation	July 10	65.75	7.21	7.12	8.01	22.43	11.15	59.55	66.40	5.74	11.91	Negative.
		July 13	56.53	7.02	7.32	10.22	25.00	11.44	50.66	57.90	7.33	14.20	Do.
		July 17	71.43	6.50	7.65	8.51	18.52	12.59	57.07	63.75	5.77	11.91	Do.
		July 20	63.49	5.52	6.50	7.87	21.67	12.78	58.26	64.44	3.54	11.05	Do.
	Average		64.30	6.56	7.15	8.65	21.90	11.99	56.38	63.12	5.59	12.27	
	Clinical	July 23	61.35	5.44	6.75	4.77	19.05	11.93	58.81	62.34	2.05	6.39	Delayed, faint.
		July 25	81.97	6.11	6.50	3.21	22.47	9.87	59.02	61.68	.71	4.94	Delayed, strong.
		July 27	68.26	7.30	10.40	2.60	19.23	10.65	60.53	68.99	.24	3.99	Delayed, medium.
		July 30	61.73	6.66	6.34	4.04	16.95	10.35	57.25	60.11	.47	5.42	Delayed, faint.
		Aug. 1	65.96	6.35	7.76	4.59	15.15	10.96	53.95	57.19	1.19	6.65	Negative.
	Average		67.85	6.37	7.55	3.84	18.57	10.75	57.91	62.06	.93	5.48	
	Subclinical	Aug. 3	66.66	6.15	6.66	5.30	18.18	11.27	54.21	58.00	2.61	7.60	Do.
		Aug. 6	58.82	5.71	7.11	6.27	15.38	13.22	55.28	59.87	2.91	8.90	Do.
		Aug. 10	69.20	6.66	7.42	6.68	16.65	14.20	53.19	57.98	4.75	9.46	Do.
		Aug. 17	62.29	6.15	7.43	6.27	19.05	10.04	61.22	66.16	2.89	8.90	Do.
		Aug. 22	78.74	4.25	7.43	10.57	15.39	11.12	62.31	71.03	5.44	14.67	Do.
	Average		67.14	5.78	7.21	7.02	16.93	11.97	57.24	62.61	3.72	9.91	

125	Normal	May 23	64.50	7.01	8.66	11.75		11.89	69.17	80.58	5.75	16.25	
		May 29	71.43	6.78	7.88	11.69		10.90	60.78	70.93	8.51	16.17	
		June 4	57.97	8.08	7.54	11.45		12.90	63.63	73.29	5.90	15.85	
		June 12	70.92	5.80	6.12	11.38		12.68	63.11	73.21	7.33	15.75	
		June 20	55.10	7.48	7.65	11.02		9.99	61.37	70.82	6.43	15.27	
		June 26	51.28	7.27	7.03	12.44		10.90	57.01	67.27	4.40	17.18	
		July 2	76.04	7.27	7.76	10.63		10.53	63.30	72.67	4.48	14.75	
		Average	63.89	7.10	7.52	11.48.		11.40	62.62	72.68	6.06	15.89	
	Incubation	July 10	61.92	7.08	7.76	10.29	20.00	11.39	64.69	73.94	5.97	14.29	Negative.
		July 13	58.48	6.66	7.64	10.04	21.98	10.96	64.12	73.09	8.75	13.96	Do.
		July 17	59.41	6.84	6.93	8.97	15.38	12.10	64.53	72.27	6.71	12.39	Do.
		July 20	47.64	6.50	6.66	9.06	19.32	12.49	60.53	67.98	5.66	12.65	Do.
		Average	56.86	6.77	7.25	9.59	19.17	11.73	63.47	71.82	6.77	13.32	
	Clinical	July 23	44.74	6.15	7.31	7.66	19.42	12.66	64.44	70.88	4.05	10.90	Negative.
		July 25	74.91	9.64	7.43	6.27	23.53	10.59	70.36	76.20	1.89	8.90	Delayed, faint.
		July 27	57.14	6.66	7.65	4.39	19.80	9.68	66.83	70.84	1.41	6.35	Do.
		July 30	60.24	5.06	6.84	2.81	17.85	10.83	58.01	60.04	.47	3.77	Delayed, medium.
		Aug. 1	62.50	6.02	8.39	1.57	15.63	10.47	57.28	58.61	.00	2.61	Delayed, faint.
		Aug. 3	60.42	4.12	8.66	1.92	15.40	10.04	55.22	56.33	.00	3.08	Do.
		Aug. 6 [2]	53.48	5.71	8.38	2.32	12.50	14.20	61.53	63.68	.00	3.61	Do.
		Average	59.06	6.19	7.81	3.85	17.73	11.21	61.95	65.23	1.12	5.60	

[2] Bull 125 was killed on this date to obtain material for pathological study as it appeared that he would not live through the night.

TABLE 2.—*Chemical constituents of the blood of five bulls in health and of four of them during subsequent anaplasmosis*—Continued

Bull no.	Period	Date	Quantity of constituent per 100 cc— In whole blood: Sugar	Phosphorus	Serum proteins	Hemoglobin	Urea	In serum, calcium	Volume of constituent— In whole blood, carbon dioxide	In serum, carbon dioxide	Whole blood: Oxygen content	Oxygen capacity	Results of Van den Bergh direct test for serum bilirubin
		1934	*Mgs*	*Mgs*	*Mgs*	*Gms*	*Mgs*	*Mgs*	*Percent*	*Percent*	*Percent*	*Percent*	
124 (control)	Normal	May 23	66.00	6.73	6.93	10.36		10.40	79.23	91.35	7.19	15.06	
		May 29	62.50	6.15	7.12	11.33		10.22	62.36	72.34	8.27	15.69	
		June 4	61.35	8.00	5.65	10.39		10.23	65.15	74.15	5.22	14.43	
		June 12	67.57	6.72	6.19	10.39		9.73	68.64	78.66	6.15	14.43	
		June 20	55.10	7.48	6.93	11.78		9.51	62.09	72.21	9.05	16.29	
		June 26	47.62	8.00	7.32	11.48		11.14	62.10	72.04	6.41	15.89	
		July 2	66.66	7.84	6.50	8.69		10.77	63.81	71.47	4.24	12.18	
	Average		60.97	7.27	6.66	10.71		10.29	66.20	76.03	6.65	14.85	
	Normal control of incubation	July 10	56.66	6.72	7.22	10.11	20.83	11.39	58.78	69.01	6.21	14.05	Negative.
		July 13	56.53	6.56	6.27	10.74	23.53	10.96	59.02	67.75	8.04	14.90	Do.
		July 17	65.57	7.21	5.84	9.04	13.42	12.35	59.92	67.29	6.02	12.62	Do.
		July 20	59.70	6.40	7.32	11.07	20.00	12.25	63.05	72.28	6.37	15.34	Do.
	Average		59.61	6.72	6.66	10.24	19.44	11.74	60.19	69.08	6.66	14.23	
	Normal control of clinical	July 23	63.09	6.15	6.58	10.22	15.38	13.29	61.62	70.37	6.81	14.20	Negative.
		July 25	71.43	6.61	6.50	11.03	15.87	10.35	59.43	68.64	6.39	15.28	Do.
		July 27	56.66	6.56	7.43	9.79	17.70	10.41	60.78	68.99	5.19	13.63	Do.
		July 30	53.19	(4)	6.27	9.65	16.07	(4)	61.05	69.27	6.62	13.44	Do.
		Aug. 1	58.82	6.20	8.39	10.33	15.63	10.96	60.35	69.10	7.38	14.48	Do.
	Average		60.64	6.38	7.03	10.21	16.13	11.25	60.65	69.27	6.48	14.21	
	Normal control of subclinical	Aug. 3	63.69	5.71	7.32	9.51	16.66	11.02	62.61	70.75	5.46	13.25	Negative.
		Aug. 6	54.79	6.15	7.43	8.78	16.66	16.16	61.53	68.91	5.07	12.27	Do.
		Aug. 10	65.57	6.15	7.42	8.80	12.50	13.44	65.40	73.25	7.36	12.30	Do.
		Aug. 17	57.80	6.66	6.93	7.60	16.00	10.19	63.29	69.62	5.33	10.76	Do.
		Aug. 22	66.66	4.22	7.07	10.39	16.66	11.34	68.64	78.94	6.15	14.43	Do.
	Average		61.70	5.78	7.23	9.02	15.70	12.43	64.29	72.29	5.87	12.60	
Average	Normal		66.54	7.34	7.66	10.88		11.19	61.70	69.69	6.73	15.08	
	Incubation		60.96	6.48	7.39	9.51	19.57	12.04	59.24	66.99	6.92	13.28	
	Clinical		62.58	6.01	7.53	4.85	17.90	10.89	56.98	60.36	1.07	5.64	
	Subclinical [3]		65.26	6.13	7.43	7.13	15.08	13.01	58.81	64.16	3.94	9.70	

[3] Averages of bulls 120, 122, and 123.

[4] Tube was broken; consequently, no analyses could be made.

The data for the normal chemical constituents show a wide range of variation, especially marked in the sugar and gases. During anaplasmosis the deviations from the normal range were slight in some experiments and were marked in other experiments. The oxygen content and capacity of the blood were greatly reduced, complete removal of oxygen occurring in the venous blood of bulls 122 and 125 and almost complete removal in bull 123. The oxygen-carrying capacity, which shows the maximum load that can be taken on in the lungs, is dependent on the hemoglobin. The data show that during the crisis of the disease the available hemoglobin was functioning at its maximum capacity. For example, in bull 122 on July 30, when the hemoglobin was 1.56, or about 15 percent of normal, the oxygen capacity was 2.59, or about 18 percent of normal. Since the carbon dioxide is carried in the serum as well as in the corpuscles this gas was not affected as much as the oxygen. However, in bull 122 during the clinical period there was an appreciable drop in the carbon dioxide content of the serum as well as in whole blood. The range of variation of sugar in the blood during anaplasmosis was in nearly all cases within that found on normal determinations. A comparison of the readings of the affected bulls with those of the normal one shows no significant changes in urea. During the clinical period the Van den Bergh direct reaction was positive in nearly all the readings. In bull 122 on August 1 it was prompt and strong, and in connection with the very low hemoglobin, oxygen capacity, and oxygen content on the same date it shows the extreme gravity of this case of anaplasmosis.

DISCUSSION

For the discrepancies pointed out previously between the morphological data of the present writers and those of Sergent and his collaborators (*14*) the following explanations are suggested: (1) The writers' cases were probably more severe than those of Sergent and his coworkers; (2) different criteria for distinguishing the various types of white cells may have been used (for example, certain cells which the writers classified as monocytes may have been classified by Sergent et al. as large lymphocytes); (3) in certain characteristics bovine anaplasmosis as it occurs in Algeria may be different from that in the United States. However, the morphological data of De Kock and Quinlan (*4*) in Africa, Livotov (*10*) in the Union of Soviet Socialist Republics, and Stephan and his collaborators (*16*) in Brazil appear to be in agreement with those of the present writers. The increase of monocytes and the left shift of the neutrophiles are in accord with current theories of immunity. For the eosinopenia no explanation has thus far been presented.

Discrepancies occur also between the chemical data of the present paper and those of previous investigators. For example, Ducloux and Cordier (*5, v. 192*) noted a ninefold increase of serum urea during the clinical period; in none of the readings by the present writers was there an appreciable increase. Nor do the data of the present writers show an appreciable increase in the blood sugar such as was noted by Ducloux and Cordier. The bilirubin analyses are in agreement. Since the data of the present paper show that the effects of the disease on the blood sugar, phosphorus, serum protein, calcium, and urea were slight, further study should be made of other constituents. For example, the proteins of the whole blood and serum

should be analyzed to determine whether there are significant changes in the albumin and the globulin fractions.

The data of previous investigators and of the present ones all show that clinical anaplasmosis is due primarily to rapid destruction of the red blood cells. This destruction causes a severe drop in hemoglobin and a consequent lowering of the oxygen-carrying capacity of the blood to a point at which it may be insufficient to maintain life. The blood stream soon becomes loaded with infected cells and debris which are converted in part into bilirubin. Most of this is removed in the bile but part of it accumulates in the serum. The reticuloendothelial system responds by removing infected erythrocytes and debris. Therefore, the outstanding factors in the behavior of the causative agent of anaplasmosis concern its ability to destroy red blood cells, and on this point the present paper does not furnish any information to indicate methods of controlling the disease. Further research is needed to determine how this destruction is accomplished, whether by hemolysins, toxins, or other agents. Within 5 to 10 days after the appearance of symptoms, the destructive agents are counteracted by products elaborated by the etiological agent, by the host, or by both combined. Precise information concerning these counteracting products is also urgently needed.

SUMMARY

This paper records the results of studies made to determine the total red- and white-cell counts, the differential white-cell counts, and the chemical constituents of the blood of cattle during anaplasmosis and health. It was hoped that the results would yield sufficient information regarding the causal agent of the disease to suggest a method of control.

Of five healthy bulls between 1½ and 2 years of age, four were injected intravenously with virulent blood of cattle affected with anaplasmosis, and the fifth was used as a control. Morphological and chemical studies were made of the blood of the five animals for 6 weeks before injections were made and during the course of the disease.

In four affected bulls, during the incubation period of anaplasmosis the readings did not change significantly from the normal.

In general, in the four bulls during clinical anaplasmosis the red-cell count dropped severely; in one case it fell below 1,000,000 red cells per cubic millimeter of blood. The white-cell count increased, the extreme reading being more than 35,000 per cubic millimeter. The differential white-cell count showed a mild lymphopenia, a marked monocytosis, a shift of the neutrophiles to the left, and a marked eosinopenia.

During clinical anaplasmosis the average hemoglobin dropped severely to 3.85 g per 100 cc of whole blood, the oxygen content to 1.07 volumes percent, and the oxygen capacity to 5.64 volumes percent. In one case during the clinical period there was an appreciable drop in the carbon dioxide content of serum and of whole blood. There were no significant changes from the normal in the sugar, phosphorus, serum proteins, calcium, and urea. During this period the serum bilirubin increased until it became detectable by the Van den Bergh direct reaction.

All of the morphological and chemical changes found in the blood of cattle having anaplasmosis were due either indirectly or directly to the rapid destruction of the red blood cells. The nature of the agents causing this destruction was not determined; hence the studies did not indicate a method of controlling the disease.

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A GENETIC FACTOR FOR THE ANNUAL HABIT IN BEETS AND LINKAGE RELATIONSHIP [1]

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INTRODUCTION

Practically all the cultivated varieties of *Beta vulgaris* L., including the sugar beet, show wide genetic variability in respect to the annual and biennial growth habit. Beets that are biennial form vegetative foliar rosettes during the first season of growth. After a period of necessary low-temperature exposure the crown buds of such vegetative beets develop aerial stems bearing spikelike inflorescences. In the annual type this stem development takes place during the first season. These annual types are commonly termed "bolters." Their occurrence is widespread and has been reported from practically all regions of the beet culture in the United States and Europe.

A review of literature indicates that a divergence of opinion exists as to the mode of inheritance of the annual character in beets. As early as 1876, Rimpau (*13*) [3] cited evidence that annual habit is dominant. In a controlled cross between the annual wild beet (*Beta patula* Ait.) and pollen from relatively biennial commercial stock, all the hybrid progeny proved to be annuals. Dudok van Heel (*3*) concluded that bolting factors are recessive. However, this conclusion was not supported by critical breeding data. Vilmorin (*14*) noted marked strain differences in bolting, but from the crosses made he was not able to define the mode of inheritance. Munerati (*10*), in 1931, contributed the first significant advance on the genetics of the bolting character. He crossed several strains which in previous tests had proved to be uniformly annual and biennial, respectively. Some of the biennials used in these crosses were selections from commercial stock, and the annuals were apparently also established from economic sugar-beet varieties. All the F_1 plants were decidedly annual, although showing a somewhat later time of blooming than the annual parent. In summing up Munerati's data which are restricted to reciprocal F_2 progenies, the observed numbers of 3,502 annuals and 1,155 biennials agree closely with the expected 3 : 1 ratio. Munerati concluded that the annual tendency is controlled by a simple Mendelian factor.

The results on the inheritance of the annual character presented in this paper were derived from crosses of one of Munerati's annual strains with several relatively biennial types selected by the writer. Aside from an extension of the simple genetical analysis of the annual habit, the chief contribution relates to a linkage between the factor for annual habit and a factor that chiefly affects hypocotyl and crown

[1] Received for publication Mar. 5, 1936; issued November 1936.
[2] The writer wishes to express his appreciation to F. V. Owen, geneticist, and Eubanks Carsner, senior pathologist, Division of Sugar Plant Investigations, for suggestions and criticism during the progress of this work and in connection with the preparation of the manuscript.
[3] Reference is made by number (italic) to Literature Cited, p. 510.

color. A foliage venation character resembling that of the common plantain (*Plantago major* L.) was also introduced in some of the crosses and gave an opportunity for a study of three contrasting characters.

RELATION OF ANNUAL HABIT TO BREEDING PROBLEMS

The removal of strong bolters in newly selected disease-resistant stocks constitutes a major breeding problem. The need for nonbolting beet varieties suitable for early planting under California conditions is shown by the results of bolting tests conducted by Carsner [4] with two lots of seed grown from the U. S. 1 variety and separated on the basis of time of maturity of the seed. Those plants on which the seed matured early were harvested June 7, 1932. This selection was given the designation V. Selection W, the later maturing lot, was harvested July 8. In a bolting test at Spreckles, Calif., with seed planted January 3, 1933, the final counts on September 6 gave the following bolting percentages: V, 77; W, 23; and the commercial brand Elite Braune, 6. The average weight in pounds per beet at harvest was: V, 1.14; W, 2.23; and Elite Braune, 2.14. In sugar percentage also selection V was the lowest. This evidence shows that in extreme cases bolting results in large losses in yield of sugar.

Results obtained by Esau (*4*) indicated that in the European variety Old Type, grown in central California, the occurrence of a relatively high percentage of bolters may have had very little influence on the final yield. It might be assumed that in this commercial brand selection had removed many of the rapid and hence most undesirable bolting types. The breeding problem now is to combine the nonbolting tendency exemplified by the best European varieties with specific disease resistance to meet conditions where the crop is grown. A demonstration of the heritable basis of the annual habit and the linkage relations to other characters would be helpful in such practical breeding procedures.

MATERIAL

ANNUAL AND BIENNIAL PARENTAL STRAINS

Annual Strain

During 1931 two beet strains [5] were tested for bolting. One of these proved to be uniformly annual, even in plantings made as late as June 25 at Salt Lake City, Utah. Figure 1 shows the annual strain in comparison with relatively biennial strains. In subsequent crosses the annual strain proved to be homozygous for both of the simple Mendelian characters—red hypocotyl-crown color and normal pinnate venation—with which the writer has worked extensively.

Biennial Strains

The biennials used for crosses are representative of similar types found in economic varieties. A brief history of these biennial strains follows:

[4] Carsner, E. Unpublished data of Division of Sugar Plant Investigations, Bureau of Plant Industry U. S. Department of Agriculture.

[5] Received from Dr. O. Munerati, Rovigo, Italy, to whom grateful acknowledgment is made.

Strains 12c21 and 042.—Both of these strains were originally selected by Carsner (*1*) for high resistance to curly top. In 1931 the writer selected from 12c21 a group of eight stecklings that were relatively nonbolting in habit. Further tests of the seed increase from these stecklings proved that the selection was a strong biennial type producing no annuals. This strain was also homozygous for both red hypocotyl color and normal venation. The original stock of 042 contained an appreciable number of bolting types. In an early planting of 1930 at Salt Lake City the 042 strain produced no bolters, but other related strains bolted as high as 50 percent. This indicates the absence of annual types in the 042 strain. This strain has yellow hypocotyl color and its leaf venation is normal.

Plantain strain.—This strain traces back to a selection from commercial Kleinwanzleben stock made by D. A. Pack, formerly associate agronomist, Division of Sugar Plant Investigations. It bred true for

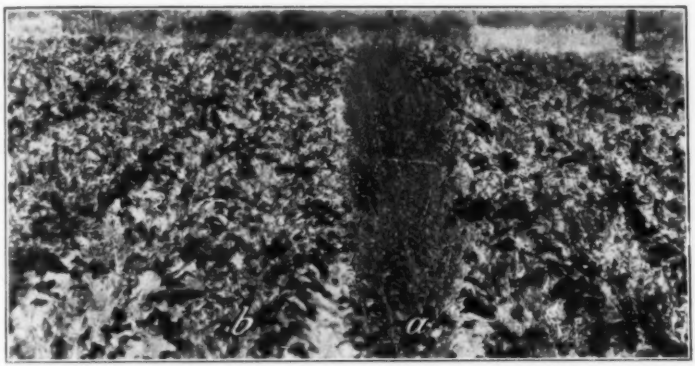

FIGURE 1.—Munerati's annual strain (*a*) in bloom, compared with his biennial type (*b*, in the single row to the left of *a*) and with other biennial strains. Planted at Salt Lake City, Utah, March 24, and photographed June 19, 1931.

a foliage type marked by semiparallel veins. The foliage venation of the strain under consideration shows prominent lateral veins that are more or less free to the base of the leaf blade, and in this respect it resembles the ribbed condition of the foliage of the common plantain, *Plantago major* (fig. 2). This selection will be designated in this paper as the plantain strain. Reselections have been extensively tested for a number of years and have shown no annuals. This type carried the recessive yellow hypocotyl-crown color.

HYPOCOTYL-CROWN COLOR CHARACTER

The red and yellow hypocotyl-crown color character, which has been mentioned under the description of the annual and biennial parental strains, produces sharply contrasted types. Keller (*8*) describes the *R* plants as having a rose or pale-red color in seedling hypocotyls. The bases of stems and petioles also may show this rose or red color. In large vegetative beets the central bud clearly shows the pigment. The writer has also been able to detect this color in most types in small buds located in the axils of leaf petioles

and seedstalks. This is a means of classifying plants in the bolting stage, in which the color intensity may be reduced.

The yellow type (*r*) lacks anthocyan pigment. The hypocotyls are green, with more or less yellow pigment. The intensity of this yellow pigmentation is apparently correlated with exposure to sunlight.

Nuckols (*11*) notes that in young sugar-beet seedlings the color of the red hypocotyl types may vary from a carmine red to light shades of pink. In the seedlings lacking anthocyan pigment the yellow

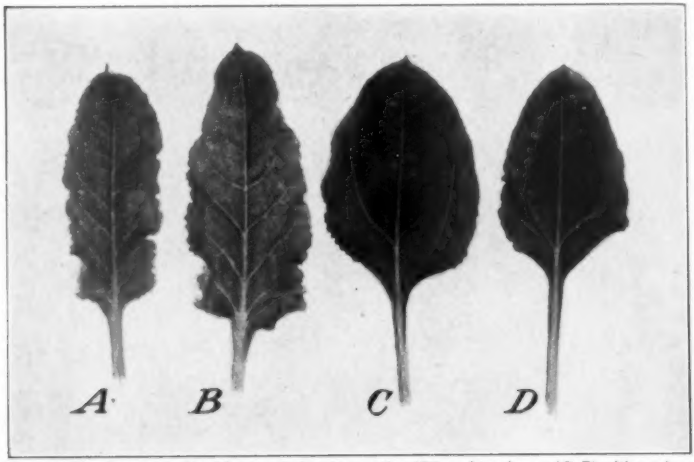

FIGURE 2.—Two leaves (*A*, *B*) with normal pinnate venation (*Pl*), and two leaves (*C*, *D*) with semiparallel venation (*pl*) typical of the plantain beet strain. Foliage taken from sib plants in backcross progeny (8 (*pl* × *Plpl*), February 1932.

coloration varies from deep orange to light shades that appear almost white. In a test of 37 varieties Nuckols found that the red and yellow color classes were apparently equal in both root yield and sugar content.

CROSSES

The following crosses and their reciprocals were made:

Cross 1, annual × 12c21.
Cross 2, annual × 042.
Cross 3, annual × plantain strain.

METHODS

CROSSING TECHNIQUE

The types crossed were highly self-sterile. Therefore the methods of distance isolation and bagging proved to be satisfactory for crossing. In the crosses made by the writer, the percentage of contamination with outside strains was very low. The bagging technique was similar to that used in corn breeding. For crosses a simple exchange of bags of the two parents yielded a satisfactory amount of seed. Since self-sterility is not complete under all conditions, the presence of selfed individuals needs to be considered. In crosses such as *rr* × *RR*, the color character shows the presence of selfs having the

r plant as seed parent and certifies the red plants as crosses. The reciprocal combination does not furnish this information immediately, and a further breeding test is required. In the combination $rr \times Rr$ a large excess of yellow types may be indicative of considerable selfing, and likewise in the $Rr \times rr$ cross an excess of reds may be due to selfs. Such marked deviations, however, occur rarely under conditions of strong self-sterility.

FIELD TESTS

Crosses between the annual type × 12c21, 042, and the plantain strain, respectively, were made in 1932. In 1933, F_1, backcross, and F_2, progenies, as well as parental strains, were tested for annual habit. This material, sown on March 23, was transplanted to the field during the last week of April. The backcross and F_2 plants were either definitely annual or biennial in character, with the exception of four plants that turned vegetative after the initiation of a seed-stalk. This material was classified for bolting on July 5, July 20, and November 11. The last two counts showed close agreement. A field planting of backcross progeny 3492 ($F_1 \times$ plantain strain) was made May 31. A count made July 10, only 40 days after planting, showed a good separation into annual and biennial types.

On April 23 and 24, 1934, several progenies and parental strains were sown in small 3-inch pots. Seedlings which had been singled were marked, but in the subsequent field test transplants and undisturbed seedlings showed no difference in the percentage of bolters. Approximately 1,000 of these potted plants were transplanted to the field on May 22 and spaced uniformly from 6 to 8 inches in the row. The material was classified on July 9 and 30. Between these two dates only one plant in all the progenies changed from the vegetative to the flowering state.

On April 26, 1934, several F_2 lots and parental strains were field-sown. The plants were thinned at random to 4 to 6 inches apart in the row. A maximum of bolting was apparently reached on July 22. A previous count on July 2, especially in progenies 3427 and 3432, showed a very large excess of vegetatives. The slow rate of bolting may be partly attributed to a severe infection of curly top to which the plants were exposed. The same F_2 lots were grown under conditions of reduced disease or in the absence of curly top, and in both cases the agreement between the expected and observed annual to biennial ratio was satisfactory.

In both the 1933 and 1934 field tests, check plantings were made of the three parental biennials, 042, 12c21, and plantain strain, respectively, and likewise of the annual type. Out of a total of 320 plants from the biennial strains tested, all remained vegetative. All of the annual parental strain, a total of 241 plants, bolted early. All 84 F_1 plants from crosses 1 and 2 proved to be annual in habit.

GREENHOUSE TESTS

By means of controlled experiments it has been shown by Garner and Allard (*6*) and Chroboczek (*2*) that lengthened photoperiods and low-temperature exposure are conditions which, if applied in proper growth stages, increase bolting in beets. Under greenhouse conditions the manipulation of temperature and light serves a useful purpose in genetic studies of growth habit.

The parental strains and the F_1 and F_2 progenies were sown outdoors in flats on August 12, 1933. On September 12, transplants were set in a greenhouse bench and spaced 3 to 4 inches apart in the row. Rows were spaced 8 inches apart. The greenhouse temperatures over the essential period of the test are given in table 1.

Shortly after the plants were set into the bench, additional light was furnished from 100-watt globes spaced 3 to 4 feet apart. From the middle of September to the middle of November these plants received an average of 8 hours of artificial light in addition to the regular daylight.

The separation between annuals and biennials was excellent. In cross 1 (annual × 12c21 biennial) a period of 60 days intervened between the appearance of the last F_2 annual and the beginning of seedstalk formation among the F_2 biennial segregates. In cross 2 (annual × 042 biennial) a period of 40 days separated the completion of bolting by the F_2 annuals and the initiation of seedstalks among the biennial segregates. In cross 3 (annual × biennial plantain strain) an average period of 35 days separated annuals and biennials. In this cross only 2 late and more or less intermediate bolters appeared in a total of 244 F_2 plants tested.

TABLE 1.—*Greenhouse temperatures for September to December 1933*

Month	Maximum	Minimum	Mean	Month	Maximum	Minimum	Mean
	° F.	° F.	° F.		° F.	° F.	° F.
September	90.2	52.4	71.3	November	76.7	48.8	62.8
October	83.6	43.7	63.6	December	73.7	43.8	68.8

In the 1933 greenhouse test, check plantings were made of the three biennial parental strains, 12c21, 042, and the plantain type. A total of 84 plants from the biennial strains remained vegetative during the entire period of the test. In contrast, 65 plants of the annual type all bolted very early.

On April 24, 1934, pot plantings of several backcross and F_2 progenies were made. Until June 21 the greenhouse plants received additional light daily from two 100-watt globes burning from 9 p. m. to 5:30 a. m. On June 21 all vegetative plants were transplanted to the field for further observation. Only 2 plants in a total of 111 classified previously as vegetatives produced seedstalks later in the field.

A small number of F_3 lines were planted October 16, 1934, in a cool greenhouse with additional light. Six red and nine yellow F_2 plants from cross 2, which had been classified in the 1933 greenhouse test as biennials, were backcrossed to yellow and biennial plants. This material was classified for bolting and color January 12, 1935.

EXPERIMENTAL RESULTS

DOMINANCE OF ANNUAL HABIT

Cross 3 (annual × biennial plantain strain) was made in 1932 by means of bagged branches on greenhouse plants. Only a small amount of F_1 seed was obtained. In a field planting of May 5 a

stand of only seven plants resulted, but all were annual in habit, red in color, and normal in venation. A comparison of the blooming stage reached by the annual parent strain and the F_1 plants showed that while the annual was past full bloom three of the F_1 plants were in full bloom and four in late bud stages.

In the 1933 test, dominance of the annual habit was clearly indicated. Eighty-four F_1 plants were grown from crosses 1 and 2 (12c21 × annual and 042 × annual), respectively. It was apparent that the F_1 plants were somewhat slower in reaching the full-bloom stage than the annual type planted in the same plot. Twenty-eight plants of strain 042 and 20 plants of strain 12c21 remained vegetative. Figure 3 shows the relative development reached by the annual

FIGURE 3.—Dominance of annual habit. Rows *a* and *b*, annual and biennial 042 parental strains, respectively; row *c*, F_1 annual plants from cross 042 × annual. Transplanted in April and photographed July 5, 1933.

and biennial parental strains and their F_1 progeny from cross 2 (042 × annual).

The 1933 greenhouse test gave F_1 results in agreement with the field test. Sixty-two F_1 plants from crosses 1 and 2 were definitely annual in habit but slower in developing seedstalks than the annual parental strain. All greenhouse plantings of the biennial parental strains remained vegetative.

Dominance of the annual habit presents a problem, especially in relation to the comparative rates of seedstalk development of the annual parent, F_1 bolters, and the annual F_2 segregates. Munerati (*10*) has called attention to the fact that the annual parent develops a seedstalk more rapidly than either F_1 plants or annual F_2 segregates. The writer has gathered some preliminary data on this question also

from material grown in the greenhouse. Spacing of plants in replicated rows was quite uniform. The measurement of seedstalk height was made when the plants of the annual parent strain were nearly all in or near the blooming stage.

The data in table 2 show that the annual strain, with a mean stalk height of 64.54 ± 2.14 cm, is significantly higher in rate of bolting than either F_1 or F_2 annuals. The coefficients of variability of annual and F_1 plants are practically equal. This indicates that with reference to the annual and biennial character the respective parental strains were uniform. However, it is expected that the parental strains could carry factors that would modify heights of inflorescence or time of blooming.

Several hypotheses may be advanced for the apparently incomplete dominance of the annual habit. The most common hypothesis would be that heterozygous *Bb* plants (*B* is the factor symbol for annual character and *b* for vegetative character) are slower in rate of seedstalk development than the homozygotes *BB*. Some F_2 data in table 2 show that this assumption does not explain all results. With some homozygous types present, one would expect the mean seedstalk height of the F_2 bolters (neglecting the vegetative segregates) to be greater than that for the F_1 annuals. However, this is not the case. In the two F_2 progenies 3430 and 3432, the seedstalk heights are 26.39 ± 1.16 and 22.08 ± 1.78 cm, respectively. The heights of the corresponding F_1 progenies are 33.87 ± 1.66 and 26.00 ± 1.45 cm, in both cases higher than those of the F_2 lots. It is also evident from the 12c21 $\times$ annual cross that F_1 and F_2 progenies are not significantly different in coefficient of variability. The F_2 progeny of the annual $\times$ 042 cross is significantly higher in variability than the F_1 progeny. However, reference to table 2 shows that a preponderance of the higher variability of the annual $\times$ 042 F_2 progeny comes from an increase of plants in the lower seedstalk heights. There is no definite indication from these data that *BB* plants have increased the average seedstalk heights to any great extent in the F_2 progenies.

A comparison of the rate of bolting of F_1 and F_2 plants needs further consideration. It is possible that F_1 plants possess on an average a greater number of favorable growth factors for height than F_2 plants. Therefore, F_1 plants may not reveal the net effect of the *Bb* factors on rate of seedstalk development. In comparison, the inbred F_2 plants may show a lower mean seedstalk height, owing to the presence of individuals with a reduced number of favorable growth factors. If a correction could be made for the opposing effects of these growth factors in F_1 and F_2, *BB* plants might prove to be faster than *Bb* plants in seedstalk development. Detailed examination of various hybrids in other plants has shown that in many instances dominance is not complete.

Considering the frequency distributions from all three F_2 progenies in table 2, it is clear that out of a total of 143 bolters not over 10 plants approximated the average seedstalk height of the annual parent (64.54 ± 2.14 cm) and that only 1 plant in the F_2 exceeded this. If *BB* F_2 plants were considerably faster in bolting than *Bb* annuals, the expectation would be that approximately one-third of the bolters in the F_2 would be near the mean seedstalk height of the annual parent.

TABLE 2.—*Distribution and variability of seedstalk height in crosses between annual and biennial types, greenhouse test, 1933*

Current no.	Strain or cross	Vegetative plants	Plants with seedstalk of indicated height												Total plants with seedstalk	Height of seedstalks		
			6 cm	14 cm	22 cm	30 cm	38 cm	46 cm	54 cm	62 cm	70 cm	78 cm	86 cm	94 cm		Mean [1]	σ	Coefficient of variability
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Cm*	*Cm*	
2240	Annual parental type	0	------	------	------	4	1	3	10	8	12	11	1	4	54	[2] 64.54±2.14	15.72±1.51	24.4±2.5
2248	F_1 (12c21 × annual)	0	------	1	2	7	9	2	------	------	------	------	------	------	21	33.87±1.66	7.59±1.17	22.4±3.6
2250	F_1 (042 × annual)	0	------	3	9	6	3	------	------	------	------	------	------	------	21	26.00±1.45	6.63±1.02	25.5±4.2
3427	F_2 (Plantain × annual)	13	5	12	5	10	7	8	4	2	------	1	------	------	54	30.52±2.33	17.09±1.64	56 ±6.9
3430	F_2 (annual × 12c21)	9	------	7	14	19	5	1	------	------	------	------	------	------	46	26.39±1.16	7.87± .82	29.8±3.4
3432	F_2 (annual × 042)	20	5	12	15	5	3	------	3	------	------	------	------	------	43	22.08±1.78	11.70±1.26	53.0±7.1

[1] The mean, standard deviation, and coefficient of variability were calculated from the ungrouped data.
[2] All errors given as standard errors. P. E.=±0.6745 × standard error.

There is no doubt that as far as initiation of seedstalks is concerned the annual habit is strongly dominant, for F_2 ratios restricted to the strains used in this study have always been 3 annual to 1 biennial. From the data presented it seems that *BB* and *Bb* types do not differ greatly in rate of seedstalk growth. It is possible that other factors for height or maturity exist which depress the rate of seedstalk development in both F_1 and F_2 while not inhibiting initiation of the reproductive phase.

INHERITANCE OF ANNUAL HABIT (CROSS 1)

Table 3 shows that the distribution of annual and biennial plants in F_2 progenies (12c21 × annual) from cross 1 is in very close agreement with the expected 3:1 ratio. It is also apparent that the

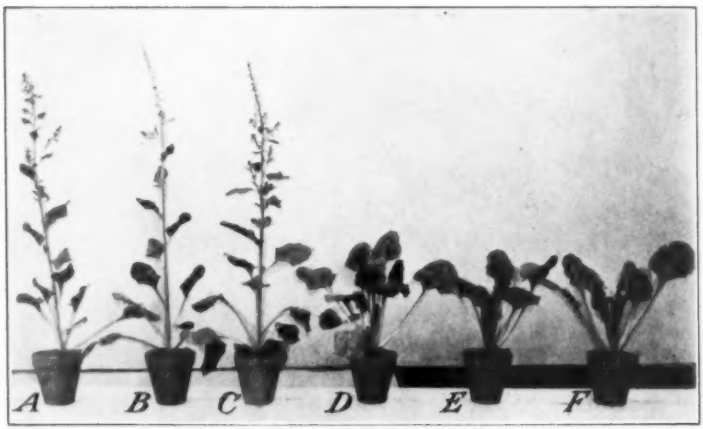

FIGURE 4.—Segregation for annual (*A–C*) and biennial (*D–F*) plants in F_2 progeny 3429 (12c21 biennial × annual), October 1933.

greenhouse lots, grown with an increased photoperiod, and the field-grown groups are practically equal in bolting percentage. Figure 4 shows annual and biennial segregates obtained from one of the F_2 progenies grown in the greenhouse.

Under greenhouse conditions the bolters in the F_2 progenies from cross 1 developed rapidly. On September 22, only 14 days after the plants were placed under light and 41 days after the seed was sown, 97 percent of all the bolters were evident. By October 18 only six more plants bore seedstalks. After that date and until December 16, when most of the plants were discarded, no more bolters appeared. The extreme vegetative nature of the F_2 biennial segregates is evident from the following test: After December 16, 10 vegetative segregates of each of the reciprocal F_2 progenies 3429 and 3430 were kept under light for further observation. On January 3, 1934, 77 days from the time the last six bolters appeared, only one plant was definitely starting a seedstalk.

TABLE 3.—*Segregation of annual and biennial habit in F_2 progeny, no. 3429 (12c21 × annual), and the reciprocal, no. 3430*[1]

Current no.	Date and location of test	Total plants	Biennial plants	Annual plants	
		Number	*Number*	*Number*	*Percent*
3429	1933, greenhouse	98	32	66	67.3
	1934, field	198	47	151	76.3
3430	1933, greenhouse	154	36	118	76.6
	1934, field	176	42	134	76.1
Total observed		626	157	469	74.9
Calculated, 3:1 ratio			156.5	469.5	
Deviation [2]			+.5	−.5	

[1] 3 stecklings each from the reciprocal F_1 lots were grown for F_2 seed in separate field isolations.
[2] $\frac{\text{Deviation}}{\text{Probable error}} = 0.07$.

INDEPENDENT ASSORTMENT OF B AND Pl

In cross 3 (plantain strain × annual) the plants differed in leaf venation as well as in growth habit. One of the biennial strains, as previously indicated, was marked by foliage venation similar to that in the common plantain. The inheritance of the plantain venation character is explained on a simple Mendelian basis, with normal pinnate venation dominant. *R* and *Pl* are independently inherited. Therefore, *B* and *Pl* should likewise show independent inheritance. The results support this expectation.

The backcross data in table 4 are in very close agreement with the calculated 1 : 1 : 1 : 1 ratio. The F_2 results reported in table 5 are not in satisfactory agreement with the calculated 9 : 3 : 3 : 1 ratio. It is not a question of linkage, for the poor fit is associated with a large plus deviation of plantain types in the annual class. The deficiency of 13 biennials is not significant. The deviation of biennials is only 2.4 times the probable error. In table 5, according to Fisher's method (*5*), the observed and calculated frequencies of annuals and biennials are compared separately in the normal and plantain classes. The fit now is satisfactory, χ^2 reducing from 10.766 to 2.851. With two degrees of freedom, *P* lies between 0.2 and 0.3.

TABLE 4.—*Independent assortment of factors B (annual habit) and Pl (leaf venation) in backcrosses of type bpl × BbPlpl*

Types backcrossed	Current no.	Annual plants		Biennial plants		Total plants
		Normal	Plantain	Normal	Plantain	
		Number	*Number*	*Number*	*Number*	*Number*
bpl × *BbPlpl*	2311, 2312, 2314	31	45	35	35	146
Reciprocal	3492	71	53	68	67	259
Total observed		102	98	103	102	405
Calculated 1 : 1 : 1 : 1 ratio		101.25	101.25	101.25	101.25	
Deviation [1]		+.75	−3.25	+1.75	+.75	

[1] $\chi^2 = 0.146$; P = between 0.98 and 0.99.

TABLE 5.—*Independent assortment of factors B (annual habit) and Pl (leaf venation) in F_2 progenies from matings of the type BbPlpl inter se*

Current no.	Total plants	Annual plants		Biennial plants		Normal [1]		Plantain [1]	
		Normal [2]	Plantain [2]	Normal [2]	Plantain [2]	Annual plants	Biennial plants	Annual plants	Biennial plants
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
2383, 2385, 2386, 3427	96, 244	50, 132	22, 64	17, 34	7, 14	50, 132	17, 34	22, 64	7, 14
Total	340	182	86	51	21	182	51	86	21
Calculated		191.25	63.75	63.75	21.25	174.75	58.25	80.25	26.75
Deviation		−9.25	+22.25	−12.75	−.25	+7.25	−7.25	+5.75	−5.75

[1] Theoretical ratio, 3 : 1; χ^2=2.851; P=between 0.2 and 0.3.
[2] Theoretical ratio, 9 : 3 : 3 : 1; χ^2=10.766; P=between 0.01 and 0.02.

LINKAGE RELATIONS BETWEEN R AND B

Backcross Data

Cross 2 was made in 1932 in a field isolation between yellow biennial 042 strain × red annual, clone 59. Seed from the two plants was harvested separately. Seventy-five F_1 plants of both combinations were tested and all proved to be annual and red in hypocotyl color. Fifty-four of these F_1 plants came from the white biennial as seed parent and all showed the presence of the *R* marker, thus certifying the cross in that particular combination.

Cross 3 was originally made in 1932 between two greenhouse plants, yellow biennial plantain venation *rbpl* × red annual pinnate venation *RBPl*. The parental plants were self-sterile. The F_1 plants proved to be annual, and all the F_1 plants backcrossed to the double recessive were found to be heterozygous for *R* and *B*. Figure 5 shows the annual parent that entered into cross 3 and a representative plant from the biennial parental strain. The plantain leaf venation of the biennial strain is evident.

Table 6 gives a summary of data from seven backcross progenies. Seed was obtained by the bagging method. The results clearly indicate linkage in the coupling phase between *R* and *B*. Out of a total of 690 plants observed, 110, or 15.94 percent, were of the recombination classes *Rb* and *rB*. This direct value is practically equal to the 16.1±0.95 percent crossing over as calculated by Owen's (*12*) product-moment method. The cross-over value calculated from the total of cross 2 was 20.0±1.60 percent, the individual values ranging from 18.0 to 25.8 percent. In comparison, the cross-over value of 13.0±1.13 percent from cross 3 was considerably lower.

The total number of annuals and biennials, 342 and 348, respectively, is in very close agreement with the expected 1 : 1 ratio, showing a deviation of only ± 3. This comparison is made in table 7.

The total numbers of observed red and yellow hypocotyl types deviate from the calculated by only ±5. There is therefore no disturbance in the ratios which may be attributed to selfing of either parent in the backcrosses. In the backcross *rb* as female there is no excess of yellow types, and in the *RBrb* female there is no indication of an excess of red-hypocotyl plants. Figure 6 shows annual and

FIGURE 5.—Parental types used for cross 3 differ in three factors: (*A*) Red annual pinnate veins (*RBPl*) × (*B*) yellow biennial plantain veins (*rbpl*).

FIGURE 6.—Annual and vegetative segregates in backcross progeny 2311 (*rb*×*RBrb*). The vegetative plant (*a*) shows the plantain venation character. Photographed July 5, 1933.

vegetative segregates from backcross progeny no. 2311 (*rb* × *RBrb*). The vegetative plant happens to be of the plantain venation type, while the three bolters are normal in venation.

TABLE 6.—*Linkage in coupling phase between factors R (hypocotyl color) and B (annual habit) in backcross progenies of the type red annual F_1 (RBrb) × yellow biennial rb and reciprocal and in F_2 progenies of red annual F_1 plants of the type RBrb crossed inter se*

BACKCROSS PROGENIES

Cross no.	Type of mating	Current no.	Red hypocotyl		Yellow hypocotyl		Total plants	Cross-over value [1]
			Annual plants	Biennial plants	Annual plants	Biennial plants		
			Number	*Number*	*Number*	*Number*	*Number*	*Percent*
2	*rb*×*RBrb*	3498	58	14	13	58	143	
	do	3499	22	8	9	27	66	
	RBrb×*rb*	3530	30	3	11	34	78	
	Total		110	25	33	119	287	20.0±1.60
3	*RBrb*×*rb*	3492	111	17	14	117	259	
	rb×*RBrb*	2311, 2312, 2314	65	12	9	58	144	
	Total		176	29	23	175	403	13.0±1.13
2 and 3	Total		286	54	56	294	690	16.1± .95

F_2 PROGENIES

2	*RBrb*×*RBrb*	3432	549	72	62	163	846	16.66± .96
		3535	99	8	8	30	145	11.50±1.92
		3536	206	17	6	34	263	9.61±1.30
	Total		854	97	76	227	1,254	14.81± .74
3	do	3427	177	17	19	31	244	17.89±1.86
		2383, 2385, 2386	64	6	8	18	96	15.41±2.74
	Total		241	23	27	49	340	17.18±1.54
2 and 3	Total		1,095	120	103	276	1,594	15.29± .67

[1] The cross-over value for the backcrosses calculated according to Owen's (*12*) product-moment correlation. The cross-over value for the F_2 progenies and the probable errors for both backcross and F_2 distributions calculated from Immer's (*7*) tables.

F_2 DATA

Table 6 gives also a summary of the F_2 linkage data between *R* and *B* derived from crosses 2 and 3. Progenies 3432 and 3427 were obtained from field isolations of three and six F_1 stecklings, respectively. Both were grown in plots distant enough from other lots to preclude contamination. The remaining F_2 progenies were obtained from F_1 plants crossed inter se by the ordinary exchange of bags.

The cross-over values, as calculated by Immer's (*7*) tables, ranged from 9.61 to 17.89 percent. Out of a total of 1,594 F_2 plants, 223 were recombinations, giving a cross-over value of 15.29 ± 0.67 percent. Eleven hundred and ninety-eight plants were annual in habit, and 396 remained vegetative. This is in very close agreement with

the expected 3 annual to 1 biennial ratio. Table 7 shows an F_2 summary of the simple 3 : 1 ratios, including also data from cross 1 (12c21 biennial × annual clone 62). The close agreement is apparent.

TABLE 7.—*Summary of segregation of annual and biennial habit from backcross and F_2 progenies, respectively*

Progenies	Annual plants	Biennial plants	Total plants
Backcrosses:	*Number*	*Number*	*Number*
Total observed	342	348	690
Calculated, 1 : 1 ratio	345	345	690
Deviation	−3	+3	0
F_2 progenies:			
Total observed	1,667	553	2,220
Calculated, 3 : 1 ratio	1,665	555	2,220
Deviation	+2	−2	0

Progeny 3427 (table 6) was also grown in the field in 1934. A total of 635 F_2 plants were classified for color and annual habit. The distribution for bolting is shown in table 8.

The excess of 47 vegetatives, which was evident in both *R* and *r* color classes, varies significantly from expectation. The failure to bolt probably lies in the fact that these plants were exposed to an early infection of curly top. The parental strains that were combined in cross 3427 were known to be highly susceptible to this destructive virus disease. Under greenhouse conditions, excluding curly top infection, the same F_2 progeny gave a normal 3 : 1 distribution for growth habit. Under both conditions, the linkage between *R* and *B* is apparent. The cross-over value of the field-grown lot under discussion was 19.66 ± 1.21 percent, as compared with 17.89 ± 1.86 percent for the greenhouse material.

F_3 Test of Vegetative F_2 Plants

In the 1933 greenhouse test, 20 plants were classified as vegetative segregates in an F_2 distribution of cross 2 (annual × 042). Fifteen of these vegetative segregates, six red and nine yellow in hypocotyl color, were backcrossed further with the 042 *rb* biennial parent strain. An average number of 52 plants per progeny was grown. Out of a total of 777 plants, only 6 bolters were found in 2 progenies. These bolters were of a decidedly slow type as compared with the annual check lot, most of which were in bloom on January 12, 1935. One of the six red F_2 plants proved to be homozygous for color (*R*). All of the whites bred true for color in F_3. The number of F_2 reds was too small to test the *R–B* linkage.

TABLE 8.—*Segregation of annual and biennial habit in F_2 progeny 3427* [1] *grown in the field in 1934*

Analysis of progeny 3427	Annual plants	Biennial plants	Total plants
	Number	*Number*	*Number*
Total observed	429	206	635
Calculated, 3 : 1 ratio	476	159	635
Deviation	−47	+47	0

[1] See table 6 for greenhouse data.

This F_3 test, with an admittedly small number of progenies, has essentially verified the classification of the F_2 plants in cross 2 as vegetatives. New crosses between annual and biennial types are available now into which a strong self-fertility factor has been introduced. This will greatly reduce the labor of securing an adequate number of F_3 progenies and should give a critical test of the *R–B* linkage.

DISCUSSION

Interest in bolting has recently been accentuated by earlier commercial planting. West of the Rocky Mountains early sowing is practiced as a means of increasing yields and especially for reducing losses from the curly-top disease. However, in early plantings beets may be exposed to conditions that tend to a marked increase in annual beets. It has been pointed out that bolting is a serious problem in California, where the occurrence of a high percentage of annuals may reduce yields. Bolters are also objectionable to the grower because their presence gives a poor appearance to beet fields. Other tangible objections are encountered in the factory process, since these annual beets, with their woody roots and high fiber or marc content, are difficult to slice. Frequently the presence of a high percentage of bolters results in a pulp of such woody quality that it is unsuitable for stock feed.

The bolting character is so highly sensitive to environmental factors that empirical and superficial selection may often be misleading. Such factors as temperature and light may induce a vegetative condition in beets that inherently possess strong bolting tendencies. For efficiency in commercial breeding operations it is important to recognize genetic factors such as that responsible for the annual habit. Up to the present time the breeding behavior of only a few beet characters has been demonstrated, but, with the great variability existing in sugar beets, genetic investigations should reveal new factors. The presence of a small number of chromosomes in sugar beets, reported by Kuzmina (*9*) to be nine bivalents, favors the charting of new factors.

The following brief summary of the results presented in this paper shows that the annual habit is dominant over the biennial type and that it is controlled by a single Mendelian factor. A total of 690 backcross plants (*Bb* × *b* and reciprocal) gave 342 annuals and 348 biennnials. A total of 2,220 F_2 plants, from matings of the type *Bb* inter se, showed 1,667 plants in the annual class and 553 biennials. Both the observed backcross and F_2 distributions are in very close agreement with the expected 1 : 1 and 3 : 1 ratios, respectively. The check plantings of annual and biennial parental strains grown under the same conditions as the backcross and F_2 progenies bred true for their respective habits. The results obtained on the inheritance of annual habit verify the conclusion reached by Munerati (*10*) that the annual tendency is controlled by a simple Mendelian factor.

It has been demonstrated that the F_1 plants and annual segregates from F_2 progenies are slower in rate of development of seedstalks than the annual parental strain. This apparently incomplete dominance of the annual habit, also noted earlier by Munerati (*10*), does not seem to be highly correlated with a heterozygous *Bb* constitution.

The hypothesis that modifying factors exert a partial inhibitory effect should be considered in any explanation of the slower rate of seedstalk development of F_1 and F_2 plants.

Keller (*8*) has established the first linkage group in beets. The cross-over value between the hypocotyl-color factor *R* and the basic-pigment factor *Y* was found to be approximately 7.5 percent. The present study has added the annual habit factor *B* to this first linkage group.

The data for the *R* and *B* linkage has been secured from two reciprocal crosses, 042×Munerati's annual, and plantain strain ×annual. Both of these crosses were of the coupling type *rb*×*RB*.

The summarized backcross data from both crosses (table 6) gave a distribution of 286 *RB*, 54 *Rb*, 56*rB*, and 294 *rb*, and a total of 690 plants. This is obviously in very poor agreement with the expectation on a basis of independent assortment of *R* and *B*. The cross-over value from the backcross data proved to be 16.1 ± 0.95 percent. The F_2 results, from a total of 1,594 plants, likewise indicate the *R*–*B* linkage, showing a cross-over value of 15.29 ± 0.67. Extreme cross-over values were found to be 9.61 ± 1.30 and 20.0 ± 1.60 percent. The approximate average cross-over value from all data is 15.5 percent.

In cross 3 (plantain strain × annual) the new factor *Pl*, with which the writer has worked extensively, was introduced. The *Plpl* factor pair determines dominant pinnate foliage venation versus a semiparallel venation resembling that found in ribbed leaves of the common plantain. *R* and *Pl* are inherited independently. Backcross data have indicated that *B* and *Pl* are also inherited independently. The agreement with the calculated 1 : 1 : 1 : 1 ratio was very close. F_2 data likewise support the independent relationship of *B* and *Pl*.

It is highly probable that the annual factor that the writer has described also operates to produce bolting in many cultivated sugar-beet varieties. The biennial plantain strain used in the present study was derived from commercial stock. The 12c21 and 042 selections with which the writer has worked are relatively biennial in habit and appear to be, so far as the bolting character is concerned, representative of similar types commonly present in sugar-beet stock derived from commercial varieties. The annual strain, as indicated earlier, is probably related to similar annual types which Munerati (*10*) has established from commonly cultivated varieties of sugar beets. It is of interest in this connection that, aside from the above-mentioned types, even more divergent forms such as table beets, mangels, and the wild beet (*Beta maritima* L.) intercross readily with sugar beets and appear to be closely related. If the possibility that these related forms have common ancestors is admitted, it follows that homologous genetic factors may exist which produce strikingly parallel variation. In more specific terms, wild beets, table beets, and sugar beets may carry a homologous bolting-factor pair such as *Bb*. The *R* and *B* linkage discussed in this paper offers one means of testing the hypothesis of an isomorphic bolting factor.

The divergent biennial strains that the writer has crossed with the annual type have given essentially identical genetic results as far as inheritance of annual habit and the *R* linkage is concerned. The apparent correspondence of the bolting character of the biennials used in this study with those commonly present in cultivated sugar-beet

varieties gives ground for the belief that the genetic results of the present investigation may apply directly to the bolting character present in commercial sugar-beet varieties.

SUMMARY

Annual versus biennial habit (*Bb*) in *Beta vulgaris* is explained on a simple Mendelian basis. The observed numbers of annual and biennial beets in backcross and F_2 distributions were found to agree closely with the expected 1 : 1 and 3 : 1 ratios, respectively. The single-factor basis for the annual character in beets confirms Munerati's results.

Annual habit is dominant. Annuals in both F_1 and F_2 progenies were slower in average seedstalk development than the plants from the annual parental strain. This indicated that dominance, although shifting strongly toward the annual side, may not be complete. The difference between *BB* and *Bb* plants in rate of seedstalk development does not appear to be very great. Other factors may exist which partly inhibit seedstalk growth of F_1 and F_2 annuals, while not suppressing the initiation of the reproductive phase.

The *B* factor assorts independently with *Pl*, the factor connected with the plantain leaf venation character. A definite linkage of *B* was noted with the common hypocotyl-crown color factor *R*. The cross-over value from all data approximates 15.5 percent. This places the factor *B* in the *R–Y* linkage group determined by Keller (*8*).

The biennials used in this study apparently correspond in their bolting character with similar types commonly present in cultivated sugar-beet varieties. It is therefore probable that the genetic results of the present investigation may find direct application to the bolting character present in commercial sugar-beet varieties.

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ZYGOSACCHAROMYCES PINI, A NEW SPECIES OF YEAST ASSOCIATED WITH BARK BEETLES IN PINES[1]

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INTRODUCTION

The present work was undertaken to identify the yeasts that occur in blue-stained wood of trees infested by certain bark beetles. The general association of yeasts and yeastlike organisms with the blue-staining fungi isolated from the wood of beetle-infested trees has been noted by Rumbold (*8*),[4] and it was with material supplied by her that this study was begun.

Cultures of yeasts were isolated from two species of beetles, *Dendroctonus brevicomis* Lec. from the Western States and *D. frontalis* Zimm. from the Southern States, received at the Forest Products Laboratory during 1932 and 1933. It was found that a heretofore undescribed zygosaccharomycete yeast was generally associated with both species of beetles and the wood of trees infested by them. Some anascosporous, mycelium-forming yeasts not described in this paper, were also frequently present.

During the summer of 1934 a more extensive study of the flora of the beetles of the Southern States confirmed the previous findings for *Dendroctonus frontalis*. In addition it was found that other bark beetles indigenous to the region, *Ips grandicollis* (Eich.), *I. calligraphus* (Germ.), and *I. avulsus* (Eich.), carry the same zygosaccharomycete.

A brief mention of the occurrence of this zygosaccharomycete has been made in a paper by Bramble and Holst (*1*). Since experimental work is being continued on the function of the yeast in relation to the blue-staining fungi, beetles, and the host, it seemed advisable to present the results of a determinative study at this time. This paper gives a technical description of the organism and a report of its known distribution.

HISTORICAL REVIEW

The first mention of the association of yeasts with blue-staining fungi and bark beetles appears to be that of Grosmann (*3*), made in 1930. She reported having isolated three nonfermenting types of yeast: (1) A budding yeast having hat-shaped ascospores arising parthenogenetically, (2) a yeast similar to the first type but forming mycelium, and (3) a type forming mycelium but anascosporous. Her first group resembles the organism described in this paper but differs in fermentation and method of spore formation.

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[2] The writer wishes to express his appreciation to the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for the cooperation which made this study possible; to E. B. Fred and W. C. Frazier, of the University of Wisconsin, for their guidance and valuable criticism; and to C. T. Rumbold, of the Division of Forest Pathology, for her generous cooperation.
[3] In cooperation with the Forest Products Laboratory, Forest Service, U. S. Department of Agriculture.
[4] Reference is made by number (italic) to Literature Cited, p. 517.

Person (*6*), in 1931, demonstrated the concurrence of yeasts and a blue-staining fungus in beetle-attacked trees, and ascribed to the aroma of the yeast fermentation in the tree the role of attracting fresh insect attacks. No description of the yeast was given, however. Shortly after this, Rumbold (*8*) reported the presence of yeasts and bacteria in cultures of *Ceratostomella pini* isolated from the wood of trees attacked by *Dendroctonus frontalis* and *D. brevicomis*. The association was so intimate that dilution plates made with the ascospores from the perithecia "showed as many bacterial and yeast as fungous colonies." As stated before, many of these yeast cultures were used in the present work.

Recently Leach, Orr, and Christensen (*5*), in a paper on the interrelation of bark beetles and blue stain, called attention to the presence of a characteristic yeast, but gave no details as to the type of yeast. Buchner (*2*) has figured a yeast that forms hat-shaped ascospores, which he found occurring with a cerambycid, *Tetropium castaneum* Paky.

Grosmann's work indicated that the yeasts are not necessary to the life of the beetle as internal symbionts, but may act indirectly by making conditions in the bark more favorable for the developing brood of insects, and, as stated by Person, act as attractants through the fermentation they produce. Preliminary work on the problem, not included in this paper, has also failed to bring to light any evidence of a direct relationship between the yeast and the beetle.

MATERIAL AND METHODS

Cultures obtained from both eastern and western species of bark beetles over a 3-year period were studied to determine whether different species might appear from year to year or whether cultures from different species of beetles varied (table 1). The same species was isolated from year to year from different beetles. A single-cell isolation made in 1932 from a *Dendroctonus brevicomis* beetle infesting *Pinus ponderosa* Dougl. in Oregon was chosen as the type culture, with which comparisons were made.

TABLE 1.—*Source of cultures of yeast associated with bark beetles*

Blue-stained host	Beetle	Inoculum	Collected in—	
			State	Year
Pinus ponderosa	*Dendroctonus brevicomis*	Beetles and wood	Oregon	1932
Do	do	Beetles	do	1933
Do	do	Wood	California	1932
P. echinata Mill	*D. frontalis*	do	North Carolina	1932
Do	do	Beetles and wood	do	1934
Do	do	do	Alabama	1934
P. strobus L	*D. valens* Lec	Beetles	North Carolina	1934
P. echinata	do	do	Tennessee	1934
P. ponderosa	*Ips oregoni* Eich	do	Idaho	1933
Do	*I. emarginatus* (Lec.)	do	Oregon	1933
P. echinata, *P. virginiana* Mill.	*I. avulsus*	do	North Carolina	1934
Do	do	do	Tennessee	1934
Do	do	do	Alabama	1934
P. echinata	*I. grandicollis*	do	North Carolina	1934
Do	do	do	Tennessee	1934
Do	do	do	Alabama	1934
Do	*I. calligraphus*	do	North Carolina	1934
Do	do	do	Tennessee	1934
Do	do	do	Alabama	1934

A medium containing 2.5 percent of Trommer's malt extract and 1.5 percent of bacto-agar was used for all the cultural studies. Malt-extract broth of the same concentration was used for the study of growth in liquid medium. Beef-extract-peptone-starch agar was used in determining diastatic action. The fermentation work was carried out in solutions of 10-percent yeast water to which the sugars were added in approximately 2-percent concentration. No special methods were used in studying sporulation, since spore formation appears after a few days on malt-agar slants. Unless otherwise noted, all incubation was at room temperature, 24° to 26° C.

Cultures from wood were obtained according to the method described by Rumbold (*7*). Beetles were cultured by removing them from freshly exposed galleries in the tree, with either a sterile brush or needle, to malt-agar slants. When fungi were present along with the yeast they could be eliminated by making transfers every 24 hours for several days. If bacteria occurred mixed with the yeasts, a few transfers on malt agar acidified to pH 2.8 to 3.0 would yield a culture of yeast only. Final purification was accomplished by dilution plating, followed by single-cell isolations according to the method of Wright and McCoy (*10*).

As previously mentioned, all cultural observations were made on malt agar or malt-extract broth. Optimum temperature was determined by inoculating 10-cc amounts of malt-extract broth with equal volumes of a suspension of young cells, counts being made after 24 and 48 hours with a Neubauer blood-counting chamber. Cell size and shape were determined in water mounts. Measurements were made with a filar micrometer.

The action of the yeast on various sugars was tested qualitatively by the Durham tube method, and quantitative determinations of the amount of sugar destroyed were made by the method of Stiles, Peterson, and Fred (*9*). The Einhorn or Smith tube was also tried initially, but the results were not so reliable as those obtained with the Durham tube.

DESCRIPTION OF ZYGOSACCHAROMYCES PINI, N. SP.

A review of the literature has revealed no named organism with which the one here described can be identified. *Zygosaccharomyces pastori*, first described by Guilliermond (*4*), bears some resemblance to it in that hat-shaped spores are produced, but the differences in cell size, cultural characters, and sexual mechanism preclude the possibility that the two are identical or even closely related. The name *Zygosaccharomyces pini* is therefore proposed.[5]

DIAGNOSIS

Zygosaccharomyces pini, sp. nov.

Cellulis globosis, ellipsoideis vel ovatis, 2.0–6.4 × 2.5–7μ (in culturis liquidis 2.9–5.5 × 3.6–7μ), gemmantibus; ascosporis 4, post copulationem heterogamicam oriundis, pileiformibus costulatis, sine costula 1.6–2.0 × 2.3–3μ.

In ligno *Pini* spp. cum *Dentroctono* et *Ipse* spp. consociatus, U. S. A. Inducit fermentationem in glucosio, fructosio, et mannosio, nec alliis saccharis vulgaribus.

[5] Type cultures have been deposited at Centraalbureau voor Schimmelcultures at Baarn, the Netherlands, and at the Division of Forest Pathology, Forest Products Laboratory, Madison, Wis.

MORPHOLOGY

In 3-day-old malt-extract agar slants or malt-extract broth, cells round, ellipsoidal, or egg-shaped; cells on slant 2.0μ to 6.4μ by 2.5μ to 7.3μ; in broth, 2.9μ to 5.5μ by 3.6μ to 7.0μ. Asexual reproduction by budding (fig. 1, *A*).

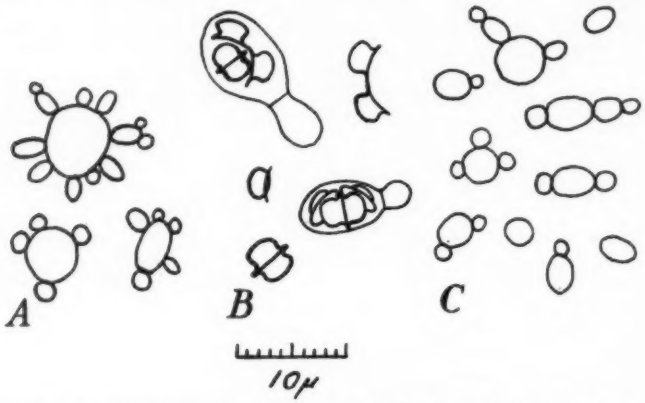


FIGURE 1.—Water mounts of *Zygosaccharomyces pini*, n. sp.: *A*, Cells from a 3-day malt-agar slant culture; *B*, ascospores and asci from older cultures; *C*, multiple budding in an anascosporous strain.

Ascospores formed after heterogamic conjugation. Sporulation occurs readily after several days on malt-extract slants. Asci contain four hat-shaped ascospores measuring, without the brim (fig. 1, *B*), 1.6μ to 2.0μ by 2.3μ to 3.0μ. Spores germinate without fusion, commonly by budding, occasionally by means of germ tube.

CULTURAL CHARACTERS

Slant cultures.—On 7-day-old slant cultures (pl. 1, *A*) growth moderate, filiform, raised, smooth, white, glistening, opaque, butyrous; medium not discolored. In older cultures growth spreading, edge finely lobate, surface may become papillate, color changes from white to fawn (pl. 1, *B*), often with white sectors remaining, perpendicular to line of inoculation.

Broth cultures.—Moderate turbidity, with slight ring present at surface after several days, no pellicle; finely granular sediment.

Giant colony.—Moderate growth, spreading, surface at first smooth, may later become papillate, irregular to lobate edge, color at first white, later fawn-colored, white radial sectors often persisting (pl. 1, *C*).

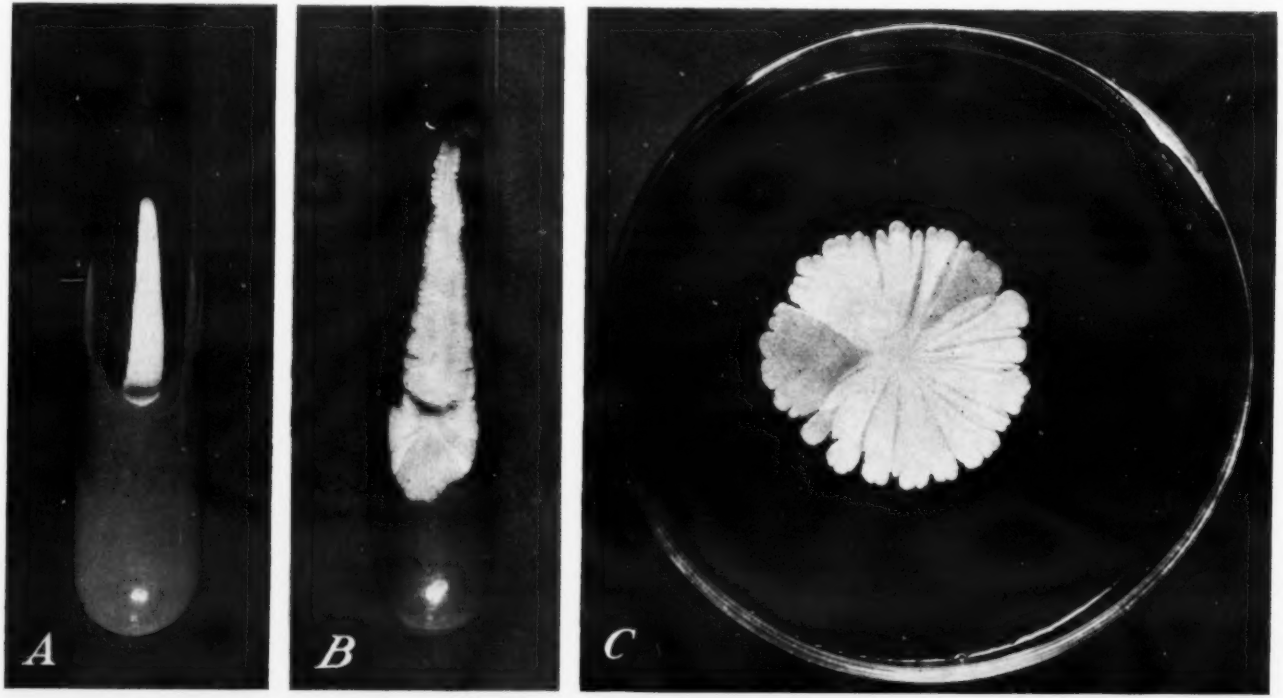
PHYSIOLOGY

Glucose, fructose, and mannose fermented with production of acid and gas; xylose, arabinose, maltose, galactose, sucrose, lactose, and raffinose not fermented. No hydrolysis of starch. Gelatin not liquefied. No change in litmus milk after 7 weeks.

Maximum temperature for growth on malt agar, 37° to 38° C.; optimum, near 30°. Maximum temperature for sporulation, 34° to 35°; growth and sporulation at 4°.

DISCUSSION

The description given for morphology and cultural characters is that of a recently isolated, freely sporulating culture. When the cultures are carried for a period of from several months to a year or more, anascosporous strains commonly begin to appear, as evidenced by the frequency of white sectors. By repeated transfer from the white sectors it is usually possible to obtain cultures that no longer produce ascospores. Even without such selection certain cultures



Cultures of *Zygosaccharomyces pini*, n. sp.: *A*, 7-day malt-agar slant culture; *B*, 40-day malt-agar slant culture; *C*, 27-day giant colony on malt agar.

ultimately lose the sexual stage and a distinct change in morphology takes place, by which such cultures may be recognized; that is, large numbers of relatively larger round to slightly oval cells 4μ to 6μ in diameter, exhibiting multiple budding (fig. 1, *C*), appear. In fact, the appearance of these cells may be taken as a forecast of incipient anascosporogeny. No change in physiology has been found to occur when a culture loses its ascospore-forming property.

There may be a variation, even with freshly isolated cultures, in the rapidity and extent of sporulation. With some cultures ascospores have not been observed until the culture is 2 to 3 weeks old, while in others after only 5 days approximately 95 percent of the cells present were ascospores. Cell size, cultural character, and fermentation properties of these various cultures were found to be in agreement, and no separation into strains appears to be justifiable on the basis of vigor of sporulation alone.

Cultures of *Zygosaccharomyces pini* possess only weak fermentative power. The yeast grows readily on glucose, fructose, and mannose, producing acid and gas in Durham tubes at room temperature, but in many cases gas does not appear until the eighth or ninth day even upon shaking, and from then on the amount increases slowly, reaching a maximum after several weeks. The fact that no acid or gas is produced cannot be taken as evidence that the sugar is not used by the organism. For example, when xylose, arabinose, maltose, galactose, sucrose, lactose, or raffinose yeast-water broths were inoculated with *Z. pini*, neither gas nor acid has produced, yet by quantitative analyses it was found that both xylose and galactose had been utilized, 25 percent of the xylose and 60 percent of the galactose having disappeared after 37 days. The remaining five sugar solutions still contained the original amount of sugars after inoculation and prolonged incubation, although the yeast grew in the solutions. It is of interest to note that the five sugars which the organism can break down, that is, glucose, fructose, mannose, xylose, and galactose, all are known to occur in coniferous woods but not as simple sugars.

SUMMARY AND CONCLUSIONS

A yeast has been found generally associated with the bark beetles *Dendroctonus brevicomis*, *D. frontalis*, *D. valens*, *Ips oregoni*, *I. emarginatus*, *I. avulsus*, *I. grandicollis*, and *I. calligraphus*. A determinative study of the organism showed it to belong to the genus *Zygosaccharomyces*, as ascospore formation is preceded by a sexual process. The formation of hat-shaped ascospores, together with the fact that of the common sugars only glucose, fructose, and mannose are fermented, make it necessary to consider this yeast as a new species. The name *Zygosaccharomyces pini*, n. sp., is therefore proposed, and a description of the cultural, morphological, and physiological characters of the species is given.

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VIABILITY OF SCLEROTIA OF SCLEROTIUM ROLFSII AFTER PASSAGE THROUGH THE DIGESTIVE TRACT OF CATTLE AND SHEEP[1]

By L. D. LEACH, *assistant plant pathologist*, and S. W. MEAD, *associate animal husbandman, California Agricultural Experiment Station*

INTRODUCTION

In the writers' studies of the agencies involved in spreading *Sclerotium rolfsii* Sacc., the cause of southern sclerotium rot of sugar beets (*Beta vulgaris* L.), circumstantial evidence has indicated that the fungus may be introduced into disease-free areas by cattle and sheep previously pastured on infected beet roots and tops. The present investigations prove that sclerotia can pass through the digestive tract of sheep and cattle without complete loss of viability. The results, however, also indicate that with proper precautions this danger can be greatly reduced or eliminated.

The passage of inert material through the digestive tract of animals has been studied by numerous investigators. Typical curves representing the rate of recovery of ferric oxide and rubber rings from cows were recently presented by Moore and Winter (*6*).[2] That some weed seeds pass through the digestive tract of animals with little or no loss of viability is well known. Several workers have investigated the possibility that plant parasitic nematodes might pass through the digestive tract of farm animals uninjured. The results of Chatin (*1*) and Triffitt (*7*) with *Heterodera schachtii*, of Leukel (*4*) with *Tylenchus tritici*, and of Wilson (*8*) with *T. dipsaci* indicate, however, that these nematodes do not survive passage through animals. Some fungus spores, on the other hand, are known to survive such passage. Ficke and Melchers (*2*) found that from a trace to 2.5 percent of corn smut spores and from a trace to 6.9 percent of sorghum smut spores retained their viability after passage.

EXPERIMENTS WITH SHEEP

PROCEDURE

The four sheep used in the first trial were confined in individual box stalls and equipped with the collection bags (fig. 1) described by Mead and Guilbert (*5*). Their ration consisted of concentrates and alfalfa hay. As soon as the animals were on full feed, 1,000 sclerotia produced from agar cultures were mixed with the concentrates and fed to each sheep at a single feeding. Thereafter only concentrates and hay were fed.

At 12-hour intervals the feces collected from each animal were washed through a series of three screens (10, 20, and 40 meshes to the inch). All sclerotia, except occasional aggregates, passed through the larger screen and were caught in either the 20-mesh or the 40-mesh

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[2] Reference is made by number (italic) to Literature Cited, p. 526.

screen. The entire residue from each screen was flushed into a large white porcelain pan with sufficient water to make a depth of 1 inch, and the sclerotia removed with forceps from the extraneous material.

After being surface-sterilized by immersion in $HgCl_2$ 1-1,000 for 45 seconds and washed in sterile water, the sclerotia were plated on potato-dextrose agar to determine their germinability. Early in these trials it was suggested that confinement of the feces in the collection bag (fig. 1) for 12 hours might expose sclerotia to a lethal concentration of ammonia. Analyses [3] revealed, however, that less than 4 p. p. m. of NH_3 was present in the confined feces. Independent investigations (*3*) have shown that the concentrations mentioned above would not be lethal to sclerotia even with much longer periods of exposure.

TABLE 1.—*Recovery and germination of sclerotia after passage through the digestive tract of sheep,*[1] *trial 1*

Time after ingestion (hours)	Sclerotia from—							
	Sheep 1		Sheep 2		Sheep 3		Sheep 4	
	Recovered	Germinated	Recovered	Germinated	Recovered	Germinated	Recovered	Germinated
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
12	4	0	1	0.0	2	0.0	12	0.0
24	56	0	72	1.4	55	5.5	60	.0
36	24	0	74	.0	86	.0	99	1.0
48	17	0	27	.0	25	.0	25	.0
60	0	.....	19	.0	11	.0	17	.0
72	0	.....	4	.0	1	.0	7	.0
84	0	.....	2	.0	1	.0	1	.0
96	0	.....	1	.0	0	.....	0	.....
108	0	.....	0	.....	0	.....	0	.....
120	.....	.....	0	.....	0	.....	0	.....
Total	101	0	200	0.5	181	1.7	221	0.5
Controls [2]	.....	90	.....	98.0	.....	98.0	.....	100.0

[1] Each animal consumed 1,000 sclerotia.
[2] Representative samples (50 sclerotia each) of original sclerotia before feeding.

RESULTS OF TRIAL 1

As indicated by table 1 and figure 2, 10 to 22 percent of the 1,000 sclerotia fed to each sheep were recovered. Most of these were passed within the first 48 hours, though one was recovered as late as 96 hours after feeding. In this trial the average recovery was 17.5 percent, of which only 0.71 percent germinated. No viable sclerotia were recovered after the 24- to 36-hour period. Many of the sclerotia recovered after the first 48 hours were broken or fragmented. This observation, together with the fact that only 17.5 percent of the ingested sclerotia were evacuated in identifiable form, led to a slaughter test to secure information concerning the remainder of the sclerotia. Animal 1 was slaughtered at the end of 108 hours, and the contents of the entire digestive tract were washed through screens and examined. As no whole or fractional sclerotia were found, apparently the remaining sclerotia had been so crushed and digested or fragmented that they passed through the 40-mesh screen.

[3] The writers are indebted to Dr. H. Goss, associate animal husbandman, for the ammonia analyses and or the preparation of the pepsin digestion solution (p. 522).

RESULTS OF TRIAL 2

In the second series the conditions of the first trial were altered in two respects. (1) Instead of the small uniform sclerotia formed in pure culture, sclerotia collected from infected sugar beets were used. The latter are often larger and more irregular (fig. 3) than sclerotia from agar cultures. (2) To simulate field-feeding conditions the sclerotia were mixed with chopped sugar beets instead of with dry concentrates as in the previous trial.

Approximately 2,500 field sclerotia were consumed by each of two sheep at a single feeding, and the feces were examined for the presence of sclerotia as in the previous trial. The chopped sugar beets produced an extremely laxative condition in the two animals and during the early part of the experimental period they failed to eat normally. As a result their evacuations were considerably retarded, so that few

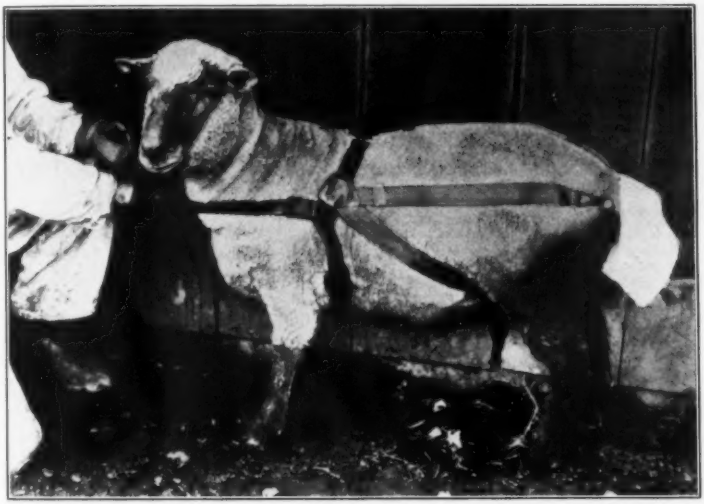

FIGURE 1.—Sheep equipped with apparatus used for collecting feces. Reproduced from California Agricultural Experiment Station Bulletin 409 (*5*).

sclerotia were recovered until the animals were again eating normally. The manner in which the feeding conditions altered the passage of sclerotia may be seen by comparing the results shown in figure 2 for sheep 1, 2, 3, and 4 (trial 1) with those for sheep 5 and 6 (trial 2).

The sclerotia recovered from the two sheep represented 13.3 and 13.5 percent respectively of those consumed, and of the recovered sclerotia 1.6 percent germinated. In this trial some viable sclerotia were evacuated 84 hours after consumption.

As an additional check on the possibility of retention of sclerotia within the digestive tract, animal 6 was slaughtered at the end of 112 hours. When the contents of the digestive tract were examined, as in the previous trial, no sclerotia were found.

The two trials indicate that from 10 to 22 percent of the sclerotia consumed by sheep are evacuated in a whole condition and that a very small percentage of these are still viable. The remainder of the sclerotia, apparently, are broken up into small fragments or digested.

TOXICITY OF RUMEN EXTRACT

Ficke and Melchers (*2*) presented evidence that contact of sorghum smut spores with the contents of the stomach of a horse for 2 hours prevented germination of nearly all the spores. Groups of 100 sclerotia were immersed in the contents of the rumen of animal 6 for 1 to 48 hours, and then tested for germinability. After 1 hour of immersion 100 percent of the sclerotia germinated; after 2 hours, 95 percent; 6 hours, 93 percent; 12 hours, 70 percent; 24 hours, 99 percent; 36 hours, 54 percent; and 48 hours, 85 percent. Similar nontreated sclerotia showed 99 percent germination. These results

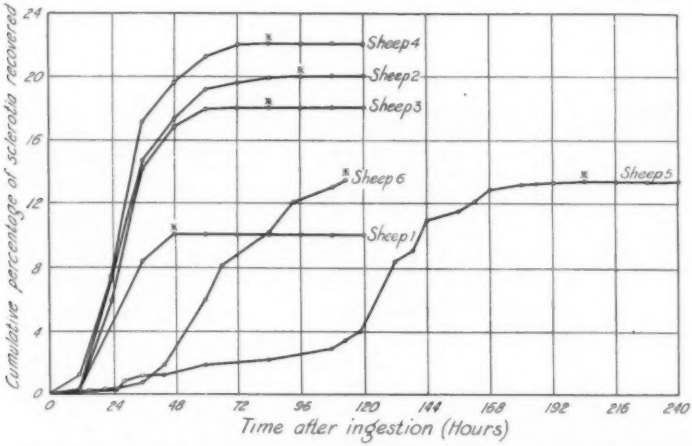

FIGURE 2.—Rate of recovery of sclerotia after passage through sheep. From 10 to 22 percent of the sclerotia fed to the animals were recovered in a whole condition. Animals 1, 2, 3, and 4 (trial 1) were fed dry concentrates and evacuated normally, whereas animals 5 and 6 (trial 2), fed chopped sugar beets, showed a retarded evacuation due to subnormal food consumption. Asterisk (*) indicates last recovery of sclerotia.

are somewhat inconsistent, and the differences are probably not significant.

PEPSIN DIGESTION

To test the effect of pepsin digestion on the viability of sclerotia, groups of 50 sclerotia each were immersed for 1 to 48 hours in a synthetic digestive solution prepared by adding 0.5 percent of pepsin to 0.5 percent of HCl and incubating for 4 to 6 hours at 40° C. The original solution had a pH value of 2.0, which remained nearly constant during the trials.

After the stated intervals duplicate groups of 50 sclerotia each were removed from the solution. One group was washed in sterile water, immersed in $HgCl_2$ 1–1,000 for 45 seconds, and then washed in three changes of sterile water before being plated on potato-dextrose agar. The other group of sclerotia was washed in sterile water and then plated without the $HgCl_2$ treatment. The results (table 2) show clearly that germination of the unsterilized groups was not inhibited by exposures of less than 12 hours in the pepsin solution. Longer exposures reduced the percentage of germination.

TABLE 2.—*Germination of sclerotia, plated with and without previous sterilization, after exposure to pepsin digestive solution at 37° C. for various periods*

Time of exposure (hours)	Germination of 50 sclerotia plated without sterilization	Germination of 50 sclerotia after sterilization with $HgCl_2$ 1–1,000 for 45 seconds	Time of exposure (hours)	Germination of 50 sclerotia plated without sterilization	Germination of 50 sclerotia after sterilization with $HgCl_2$ 1–1,000 for 45 seconds
	Percent	*Percent*		*Percent*	*Percent*
0 (controls)	100	100	12	98	0
1	100	100	24	86	36
2	100	92	36	64	0
6	100	74	48	32	2

Although the germination of normal sclerotia was not influenced by surface sterilization with $HgCl_2$, sclerotia weakened by immersion of 12 hours or more in the digestive solution were almost completely prevented from germinating by the $HgCl_2$ treatment.

These results suggested that surface sterilization with $HgCl_2$ may have been partly responsible for the low percentage of germination of sclerotia recovered from sheep feces collected during trials 1 and 2. To test this hypothesis trial 3 was conducted in March 1935, with one sheep being fed 2,500 sclerotia produced on carrots in laboratory moisture chambers. These were mixed with a single feeding of dry concentrates and fed as in the first trial.

RESULTS OF TRIAL 3

Collections were made at 12-hour intervals, and the sclerotia recovered as in previous trials. Instead of being surface-sterilized, however, the sclerotia were plated without further treatment on moist, finely screened, unsterilized peat soil in Petri dishes and incubated at 30° C. After having been plated, the sclerotia were kept under observation for at least 5 days unless germination occurred within a shorter period. The rate of recovery shown in table 3 is comparable to that obtained in the previous trials. Of the 2,500 sclerotia consumed, 13.3 percent were recovered within 60 hours, and no whole sclerotia thereafter. Of the 333 sclerotia recovered, however, 50 or 15.0 percent germinated when tested by the revised method, whereas the control sclerotia showed 58 percent germination. The percentage of germination in this trial, being considerably higher than that obtained in the first two trials, suggests that the number of viable sclerotia evacuated by the animals in the first two trials may have been considerably larger than was indicated by the figures.

TABLE 3.—*Recovery and germination of sclerotia, plated without surface sterilization after passage through the digestive tract of a sheep, trial 3* [1]

Time after ingestion (hours)	Sclerotia recovered	Germinated		Time after ingestion (hours)	Sclerotia recovered	Germinated	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
12	77	30	39.0	60 [2]	5	0	0.0
24	162	18	11.2				
36	65	2	3.8	Total	333	50	15.0
48	24	0	.0				
				Controls [3]			58.0

[1] The animal consumed 2,500 sclerotia.
[2] No recoveries at 72, 84, 96, or 108 hours.
[3] 100 sclerotia, a representative sample of original sclerotia before feeding.

EXPERIMENTS WITH CATTLE

Approximately 15,000 sclerotia collected from infected sugar beets and mixed with barley, wheat bran, and chopped alfalfa hay were fed at a single feeding to a Jersey cow. Samples of feces were screened to determine the earliest evacuation of sclerotia. None was found at 12 hours, but at the end of 24 hours a few were recovered. During the next 12 hours 226 sclerotia (fig. 3) were evacuated, of which 16, or 7.1 percent germinated despite the fact that they were subjected to $HgCl_2$ surface sterilization before plating. During the next 5 days small samples of feces were analyzed and some sclerotia recovered each time, but with a gradual decrease in number. Beginning at 156 hours after the sclerotia were consumed, all the feces were examined during the next 4 days, but no sclerotia were recovered.

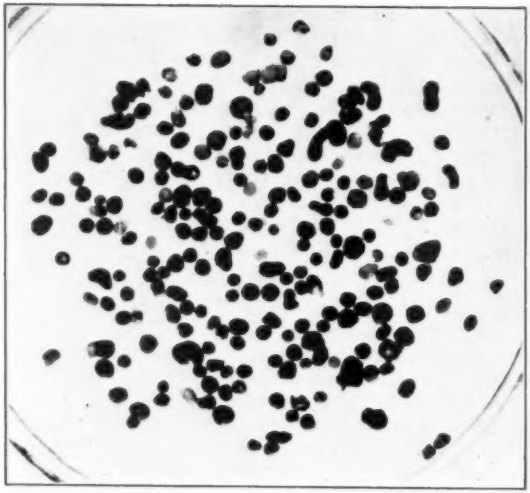

FIGURE 3.—Sclerotia of *Sclerotium rolfsii* recovered from a 12-hour collection of feces from a cow. Note that some have been broken by passage through the digestive tract. Of these 226 sclerotia, 16 (7.1 percent), germinated when plated on agar. × 1.6.

With this qualitative information in mind, another test was arranged to secure more definite information on the rate of passage, the length of retention in the body, and the percentage germination of scleortia recovered during each 12-hour period.

Approximately 14,500 field sclerotia, mixed with feed as in the preliminary trial, were offered to a second Jersey cow. Only a small portion of the feed, estimated to contain 3,000 sclerotia, was consumed at the first feeding; the remainder, containing about 11,500 sclerotia, was eaten 12 hours later. Each 12-hour collection of all feces was

thoroughly mixed, and aliquot samples consisting of 25 percent of the mass were taken for examination except when the weight exceeded 20 pounds, in which case 12.5 percent was taken. All sclerotia recovered were surface-sterilized with $HgCl_2$ and tested for germinability.

The number of sclerotia recovered, the approximate number evacuated, and the percentage of germination are presented in table 4.

TABLE 4.—*Recovery and germination of sclerotia after passage through the digestive tract of a cow*

Time after ingestion [1] (hours)	Sclerotia recovered	Germinated		Approximate number evacuated [2]	Time after ingestion [1] (hours)	Sclerotia recovered	Germinated		Approximate number evacuated [2]
	Number	*Number*	*Percent*			*Number*	*Number*	*Percent*	
12	1	0	0.0	4	132	5	0	0.0	20
24	85	21	24.7	340	144	1	0	.0	4
36	180	13	7.2	720	156	0			0
48	205	21	10.2	820	168	0			0
60	55	0	.0	220	180	1	0	.0	4
72	162	0	.0	648	192–228 (4 samples)	0			0
84	93	0	.0	744					
96	35	0	.0	280	Total	851	57	6.7	4,028
108	19	2	10.5	152					
120	9	0	.0	72	Controls [3]			88.4	

[1] Approximately 14,500 sclerotia were consumed, 20 percent of which were consumed at the first feeding and the remainder at the end of the first 12 hours.

[2] Obtained by multiplying the number of sclerotia recovered (column 2) by 4 or 8, according to the fractional part of the feces examined.

[3] 287 sclerotia, a representative sample of original sclerotia before feeding.

About 27.8 percent of the 14,500 sclerotia consumed were evacuated in a whole condition. Of those recovered, 6.7 percent germinated. On the other hand, 11.7 percent of the sclerotia recovered during the first 48 hours germinated, whereas only two viable sclerotia were recovered thereafter. At the time of these trials the deleterious effect of surface sterilization with $HgCl_2$ on weakened sclerotia was not known.

SUMMARY AND CONCLUSIONS

Sclerotia of *Sclerotium rolfsii* were added to the ration of seven sheep and two cows and their excreta examined for the presence and viability of sclerotia. From 8 to 28 percent of the total consumed were evacuated in a whole condition.

Examination of the contents of the digestive tract of two slaughtered sheep indicated that the remainder of the sclerotia were digested or fragmented, since no whole sclerotia were retained by the animals after a period of 5 days.

It was found that from 0.7 to 15.0 percent of the evacuated sclerotia retained their viability. No viable sclerotia were evacuated by sheep later than 84 hours and none by cattle later than 108 hours after ingestion.

Evidence was obtained that surface sterilization with $HgCl_2$ reduced the germination of weakened sclerotia, although sound sclerotia were not affected.

Under normal feeding conditions most of the living sclerotia were excreted within 48 hours after consumption. Subnormal food consumption retarded the evacuation of sclerotia but did not prolong the period in which viable sclerotia were excreted.

Sclerotia immersed in the liquid contents of sheep rumen were still viable at the end of 48 hours. A pepsin digestive solution reduced but did not entirely prevent germination within the same period.

These investigations prove conclusively that sclerotia can pass through the digestive tract of cattle and sheep without complete loss of viability.

The total number of sclerotia that could be transported by the movement of animals would probably not be large as compared with the quantities now occurring in infested fields. Evidently, however, cattle and sheep may introduce into uninfested fields a sufficient quantity of the parasite to produce serious losses in future plantings.

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REPRODUCTIVE CAPACITY OF FEMALE RATS AS AFFECTED BY KINDS OF CARBOHYDRATES IN THE RATION [1]

By C. H. WHITNAH, *associate food analyst, Department of Chemistry,* and RALPH BOGART, *graduate research assistant, Department of Animal Husbandry, Kansas Agricultural Experiment Station*

INTRODUCTION

When experimental rats used in a study of galactose metabolism [2] failed to reproduce normally, it was decided to determine the character of the abnormality and also, if possible, the relation of the carbohydrate in the ration to the reproductive capacity of the rat.

LITERATURE

The effects on reproduction of moderate amounts of sucrose and lactose in the rations of rats have been reported by several workers. Mitchell (*6*) [3] found that lactose, when constituting not more than 30 percent of the ration or 50 percent of the total carbohydrate in the ration, did not interfere with growth or well-being. Skinner, Van Donk, and Steenbock (*8*) found that ovarian function, as measured by length and regularity of oestrus cycles, was improved by the addition of 10 percent of sucrose to a ration of mineralized milk. Keil, Keil, and Nelson (*4*), however, reported interference with reproduction as a result of the addition of 5 percent of sucrose to a differently mineralized milk ration.

Evans (*3*) stated that a lack of vitamin E has no effect on the ova or ovarian tissue. In a comparison of vitamin E deficiency with that of vitamins A and B, he stated that vitamin A deficiency is characterized by a continuous cornified smear.

Smith and Engle (*10*) found that anterior pituitary transplants stimulated the ovaries of immature and mature rats, producing an excess of follicles. Ovulation occurred in the young animals. In older animals there were some follicular cysts, and there was also some luteinization. The cysts were sometimes twice the size of normal follicles. Engle and Smith (*2*), in a study of corpus luteum formation in pituitary-stimulated animals, found that follicles did not rupture, but formed cysts which transformed directly into corpora lutea. Sometimes eggs were found "trapped" as a result of luteinization of the follicular wall. Smith (*9*) observed that hypophysectomy of the rat resulted in a regression of the ovaries. If corpora lutea were present, they persisted for a long time. Transplants of anterior pituitary stimulated these regressed ovaries to renewed activity but follicular and lutein cysts resulted. Casida and Hellbaum (*1*), using 21- to 25-day-old rats, produced large follicles and

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[2] These animals were under the care of G. N. Woodruff of Kansas State College.

[3] Reference is made by number (italic) to Literature Cited, p. 532.

corpora lutea by injections of extracts from adrenal glands of horses. They obtained ovulation in about 50 percent of the treated animals. They made no attempt to determine whether this effect was direct or indirect. Moore and Price (7) concluded from their work that the only gonad-stimulating hormones are hypophyseal or hypophyseallike substances.

MATERIALS AND METHODS

The rations fed were designed to be adequate in every known respect. One group of rations contained a minimum amount of galactose while the other group contained lactose which provided a liberal supply of readily available galactose.

The regular experimental rations (designated according to the characteristic carbohydrate) are shown in table 1.

TABLE 1.—*Composition of experimental rations*

Food product	Sucrose	Lactose	Starch
	Percent	*Percent*	*Percent*
Yeast	5	5	4
Osborne and Mendel salt mixture	4	4	5
Cod-liver oil	1	1	1
Casein	20	20	20
Butter oil	20	20	20
Starch	30	30	50
Sucrose	20		
Lactose		20	

Commercial lactose was used for all but one group of lactose-fed rats. The substitution of Mallinkrodt's "analytical reagent" lactose for this group produced no change in the results. No further attempt was made to differentiate lactose from factors which might be associated with it as the important constituent of the ration.

These rations were fed to many groups of rats during a period of more than 2 years. The early groups included several females for which the reproductive record is not complete enough to justify detailed description but which furnished the incentive for the detailed records set forth in this paper.

Some of the rats were mated in order to test fertility. Ages at which the vaginas opened were determined. Microscopic observations were made on the ovaries of some animals, and the uteri of these animals were examined macroscopically. Serial sections of the ovaries were made for microscopic study. In some cases one ovary and part of one horn of the uterus were removed by laparotomy. Since little difference was noted between the remaining ovaries of these animals and ovaries from other animals receiving the same feed, they have been included in the appropriate age and feed groups.

OBSERVATIONS

Seven animals fed lactose, five fed sucrose, and two fed starch were kept with fertile males for 40 days or until pregnancy was detected. During the time of this test all the animals were changed from the experimental rations to the regular stock ration. The results are presented in table 2.

TABLE 2.—*The effect of feeding various carbohydrates on the reproductive ability of rats*

Animal no.[1]	Carbohydrate fed	Age of animal		Size of litter	Condition of young
		When placed with male	When litter was born		
		Days	*Days*	*Number*	
70a	Lactose	79	107	10	Normal.
78a	do	79	105	5	All died within 3 days.
84b	do	79	102	7	Normal.
90b	do	79	105	5	Do.
121c	do	56	80	4	1 died, 3 normal.
125c	do	56	93	8	Normal.
129d	do	48	85	8	Do.
1	Sucrose	80		0	
2	do	70		0	
3[2]	do	70	111	3	2 died, 1 weakly.
87b	do	79		0	
119c	do	56	80		Abortion.
123c	Starch	56	94	10	Normal.
127c	do	48	87	7	Do.

[1] The animals having like letters were litter mates.
[2] After producing 1 litter, this female was kept with a fertile male for 90 days without producing another litter.

As table 2 shows, each lactose- and starch-fed animal reproduced. All these litters, except possibly one born to a lactose-fed female, were normal. The one sucrose-fed female which produced a live litter was afterward sterile.

The age at which the vagina opened and the type of vaginal smears were determined for 17 sucrose-, 4 starch-, 6 stock-, and 18 lactose-fed animals. The results are presented in table 3. The sucrose-fed

TABLE 3.—*Effect of feeding various carbohydrates on age at which vaginas of rats opened, and types of smears secured*

Ration fed	Animals	Age at which vagina opened			Standard deviation	Vaginal smear record
		Youngest	Oldest	Average		
	Number	*Days*	*Days*	*Days*		
Sucrose	17	32	51	38	4.8	1-3 cycles, then dioestrum.
Starch	4	45	53	49	3.8	Not observed.
Stock	6	51	60	55	3.4	Normal cycles.
Lactose	18	45	64	59	6.7	Do.

animals showed sexual activity, as measured by the age at which the vaginas opened, earlier than any of the other groups. There is considerably less than 1 chance per 1,000 that this difference is due to chance. The smears of the sucrose-fed animals, however, showed only a few oestrus cycles. The lactose-fed animals were slightly late in reaching sexual maturity but showed normal cyclic changes in the smears. The starch- and stock-fed groups consisted of only a few animals, but these showed no abnormalities. They closely resembled the lactose group.

The observations made on the ovaries of the animals fed sucrose and those fed lactose are shown according to age groups in table 4.

TABLE 4.—*Effect of feeding sucrose and lactose in relation to microscopic observations of the ovaries of rats in various age classes*

Animals (number)	Age	Carbohydrate fed	Corpora lutea	Luteinization of follicles	Atresia [1]
	Days				
3	37	Sucrose	+	+	−
2	37	do	−	+	−
1	37	Lactose	−	−	+
5	44–47	Sucrose	+	+	−
2	44–47	Lactose	−	−	+
10	64–72	Sucrose	+	+	−
10	64–72	Lactose	[2] +	−	+
6	64–72	do	−	−	+

[1] Atresia indicates that follicles are so degenerated that they should soon disappear.
[2] 3 animals in this group had fresh rupture points in some of the follicles. Eggs were found in the Fallopian tubes of 2.

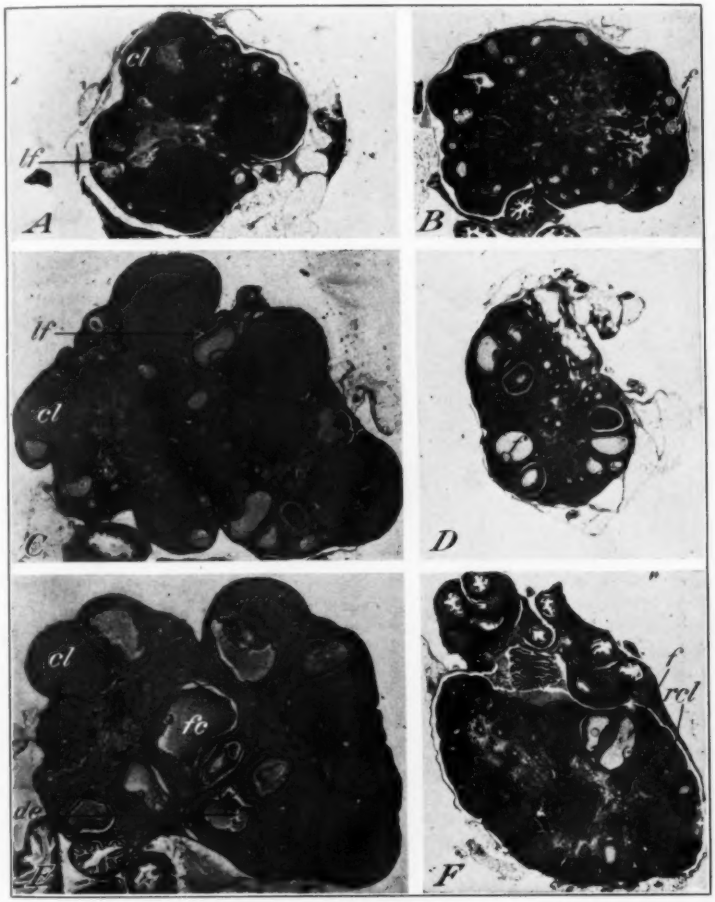
Photomicrographs of typical sections from ovaries of sucrose- and lactose-fed animals of each age group are shown in plate 1, *A* to *F*. These sections all have the same magnification, but they do not represent the relative sizes of the ovaries from different animals.

The presence of luteinized follicles in the sucrose-fed animals of all age groups would account for the lack of atretic disappearance of follicles. Luteinization of immature follicles would also explain the failure of the follicles to rupture.

As table 4 shows, the sucrose-fed animals were precocious. Corpora lutea were found in the ovaries of some of these animals at 37 days of age (pl. 1, *A*). In sharp contrast to this, the lactose-fed animal of this age was practically in an infantile sexual condition. Small follicles only were present in the ovary (pl. 1, *B*).

At 44 to 47 days of age similar differences existed as in the 37-day-old animals but they were even more striking. The sucrose-fed animals showed many more corpora lutea, luteinization of the larger follicles, and only a few small follicles (pl. 1, *C*). There was some indication that the follicles were being transformed into corpora lutea without a rupture occurring; at least no rupture points were observed. Some of the large luteinized follicles contained eggs that were degenerating. The lactose-fed animals, on the other hand, contained ovaries with follicles of all sizes (pl. 1, *D*). There was no luteinization of follicles among lactose-fed animals.

In the age group 64 to 72 days, many large corpora lutea were found in the ovaries of the sucrose-fed animals. There were no cyclic ages of corpora lutea, and no small follicles were present. Some cysts due to luteinization of the large follicles were found, but none of the corpora lutea showed signs of degenerating as would be expected if normal cycles were occurring (pl. 1, *E*). There was considerable variation among the lactose-fed animals. Some had not yet ovulated, showing some indication of retardation. In most cases, however, fresh corpora lutea and normal follicles of different sizes were found (pl. 1, *F*). In some of the lactose-fed animals the rupture points were visible, and eggs were found in the tubes of two animals. More than one age of corpora lutea was observed in the ovaries of some animals. There was no luteinization of follicles.



A, Left ovary from a 37-day-old sucrose-fed rat, showing large corpora lutea (*cl*) and small luteinized follicles (*lf*). *B*, Left ovary from a 37-day-old lactose-fed rat, showing many small follicles (*f*). There is no luteinization of any follicles. Some atresia is present. *C*, Right ovary from the same animal as *A*, at 45 days of age. A sucrose-fed rat, showing large corpora lutea (*cl*) and luteinized follicles (*lf*). No normal-appearing follicles of any size. *D*, Right ovary from the same animal as *B*, at 45 days of age. A lactose-fed rat, showing follicles of different sizes (*f*). No corpora lutea and no luteinization of follicles. *E*, Ovary from 70-day-old sucrose-fed rat, showing many corpora lutea (*cl*) and luteinized follicles, some containing degenerating eggs (*de*). Some follicles are so heavily luteinized that cysts have resulted (*fc*). *F*, Ovary from 70-day-old lactose-fed rat, showing follicles of different sizes (*f*) and recent corpora lutea with blood clots (*rcl*). There is no luteinization of follicles.

The uteri of the sucrose-fed animals of all age groups were generally larger than those of the corresponding lactose-fed animals. Some were distended with fluid and some were highly vascular.

Ovaries of three lactose-fed animals were observed about 30 days after the animals had given birth to litters. The normal cyclic ages of corpora lutea were present. Ovaries of four other animals aged about 200 days were also observed. These had been on a sucrose ration until about 70 days of age. Afterward they had been on the regular stock ration. The ovaries were made up almost entirely of masses of corpora lutea and follicular cysts. No evidence of cycles was seen, and there was indication of the cysts transforming into corpora lutea.

DISCUSSION

With our present knowledge of nutrition it is difficult to understand how the replacement of either the 20 percent lactose, or 20 of the maximum 50 percent starch, with 20 percent sucrose in the ration could have produced the observed sexual precocity and luteinization of follicles. A few instances have been reported in which sucrose in the ration was associated with abnormal reproduction. The observed abnormalities, however, were not linked with either abnormal ovarian structure nor with any pituitary disturbances. The absence of galactose might possibly be associated with the results produced by the sucrose ration. Sorensen and Haugaard (*11*) have shown that casein contains a small amount of galactose. It is possible that the additional cornstarch contained sufficient galactose to meet the need and make possible the observed relations of the three rations studied. It might, on the other hand, be assumed that the sucrose affected some gland which secreted a gonad-stimulating substance. Since most work indicates that the pituitary is the only gland which could have produced this effect, there is the possibility that the sucrose either directly or indirectly affected the activity of the pituitary.

The results obtained were similar to those produced by pituitary implants (*10*). One difference, however, is that no excessive number of follicles or corpora lutea were present. The luteinization of follicles and formation of cysts and the persistence of the corpora lutea were more like the results produced by implanting pituitaries into hypophysectomized rats (*9*). The transformation of follicular cysts into corpora lutea is similar to results obtained by Engle and Smith (*2*). The similarity of the ovarian abnormalities in the writers' animals to defects noted by other workers when the pituitary function was altered is strong evidence that some pituitary disturbance existed.

The possibility that a vitamin deficiency might have brought about the abnormalities noted is very small. The symptoms observed were not the characteristic symptoms of any vitamin deficiency. The smear record and ovarian observations are far from indicating a deficiency of either vitamin A or E.

When the mixture of purified amino acids recently announced (*5*) becomes available as a substitute for proteins, it may be practical to determine more definitely what characteristics of the carbohydrates studied are associated with the results secured in this investigation.

SUMMARY

In this study of the relation of the carbohydrate in the ration to the reproductive capacity of rats, three carbohydrates were fed, sucrose, lactose, and starch.

The sucrose-containing ration was found to be inadequate for normal reproduction in rats, even when replaced during adult life by a normal ration.

Rats receiving the sucrose ration were precocious in sexual development.

Extreme luteinization of follicles and persistence of corpora lutea were characteristic of the ovaries of the sucrose-fed animals. This adequately explains the failure of these animals to reproduce.

The ovarian abnormalities of the sucrose-fed animals indicate pituitary disturbances.

Animals fed the lactose- and starch-containing rations reproduced normally and had normal ovarian structures.

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THE DISTRIBUTION AND CONDITION OF THE POTASSIUM IN A DIFFERENTIALLY FERTILIZED HAGERSTOWN CLAY LOAM SOIL PLANTED TO APPLE TREES IN CYLINDERS[1]

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INTRODUCTION

In an earlier paper (*19*)[2] the writer reported the utilization and recovery of nitrogen, phosphorus, and potassium by apple trees grown in metal cylinders for a period of 6½ years. These trees received (each spring) during the last 3 years of growth different combinations of the pure salts sodium nitrate, monocalcium phosphate, and potassium sulphate. The ratio in which nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) were absorbed from the added salts by the trees which gave optimum growth and reproduction, viz, those growing in the NPK- and NP-treated cylinders, was 3.0:0.3:1.5, whereas the ratio in which these same fertilizer entities were applied was 3:8:4. This latter ratio is based on current orchard practice. Because of the great divergence in these ratios studies were made to determine the distribution and condition of the nitrogen, phosphorus, and potassium in the soil at the end of the experiment. The results as they relate to nitrogen and phosphorus have already been reported (*20*, *21*). The corresponding studies were made to determine the distribution and condition of the nitrogen, phosphorus, and potassium in the soil at the end of the experiment. The results as they relate to nitrogen and phosphorus have already been reported (*20*, *21*). The corresponding data for potassium are given in the present paper.

Although both phosphorus and potassium are "fixed" by soils, the solubility relations of these elements are markedly different. The phosphorus concentration of the soil solution of a particular soil varies over a relatively small range, whereas that of potassium fluctuates over a wide range even when the soil neither receives applications of potassium salts nor suffers loss by leaching or removal by the plant.

In this paper all results have been calculated to and reported as the oxide K_2O, commonly designated potash. Occasionally, in the text, reference is made to the element potassium where the use of the ionic form appears to be the more suitable. The reader, however, should keep in mind that all values given in the tables are expressed as the oxide K_2O.

METHODS

THE FIELD PLAN

Since a detailed plan of this experiment has already been presented (*1*, *20*, *21*), only an outline of the procedure will be given here. A virgin soil (*18*) of Trenton formation, formed by the weathering of

[1] Received for publication Feb. 6, 1936; issued November 1936. Paper no. 736 of the Journal series of the Pennsylvania Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 545.

limestone, was used. This soil is designated throughout as the "original" soil, and represents the condition of the soil before the experiment began. The method of excavation, the preparation and mixing of each horizon, and the filling of the cylinders have been described in detail (*18, 20*) and also the method of sampling the soil before and after the experiment (*21*).

The sources of the potassium in this Hagerstown soil are the mixed feldspars, micas, and tourmalines. The silt fraction contains a preponderance of microcline with some quartz; whereas the fine sand (0.1 to 0.5 mm) consists of 50 percent of mixed feldspars and 35 percent of quartz. Orthoclase is present in very small amounts and is highly kaolinized. No fresh unaltered samples of orthoclase exist in this soil (*18*).

For the first 2 years after the trees were planted the culture system was uniform in all cylinders. It consisted of green manuring with buckwheat and rye principally. Commencing with the third year, the system was differentiated as follows: Half of the cylinders were kept under a system of clean cultivation. These cylinders are designated "cylinders under cultivation." Half the cylinders were seeded with a mixture of bluegrass and timothy. These cylinders are designated "cylinders under sod." The schedule of applications of potassium sulphate is given in table 1.

TABLE 1.—*Schedule of potassium sulphate applications with potash equivalent*

Date applied	K_2SO_4	K_2O equivalent
	Grams	*Grams*
Apr. 18, 1925	293	158.45
May 3, 1926	147	79.49
June 7, 1926	147	79.49
May 5, 1927	293	158.45
May 18, 1927	147	79.49
Total	1,027	555.37

The total amount of potash added in the fertilizer during the whole period of the experiment was, as indicated above, 555.4 g. In addition, 25 g of potash was added in the form of cover crops of all cylinders up to the period of differentiation referred to in the last paragraph. An additional 15 g of potash was added after this period, but to the cylinders under cultivation only. The purpose in making these relatively large applications of fertilizers was to saturate the fixing capacity of the 0- to 7-inch layer of the original virgin soil with respect to phosphoric acid. The amounts of nitrogen and potash applied were porportionally increased so as to maintain the ratio 3:8:4.

LABORATORY EXPERIMENTS

The method of approach in determining the condition of the potassium was to find, in addition to the total potassium, the amounts removed by continuous leaching with three solvents, namely, (1) freshly boiled distilled water, (2) M/2 ammonium acetate, and (3) 0.2 N nitric acid. A few experiments were also carried out with 1-percent citric acid, but these experiments are not recorded inasmuch as no additional information was obtained by this reagent.

The total potash (K_2O) was determined in the three horizons of all soils by the J. Lawrence Smith method *(22)*, a 0.5 g charge of finely pulverized soil being used. The detailed results for total potash are not recorded for reasons which will be apparent later (p. 538).

The continuous leaching experiments were conducted on 10 g charges of soil in the same manner and with the same apparatus that was described and illustrated in a preceding paper (*21*). The temperature of the laboratory during the experiments varied between 75° and 85° F. Preliminary experiments showed that the increase in the amounts of potash extracted per 2° rise in temperature was equivalent to 0.9 percent of that extracted at 75°. This is relatively a negligible increase. The rate of percolation was approximately 1 drop in 90 seconds.

The potash content of the leachates was determined by three methods. When the amounts were sufficiently large for gravimetric procedure the potassium was precipitated and weighed as the chloroplatinate (*2*) after the removal of silica, iron, alumina, calcium, and barium. When, however, the amount of potassium extracted reached values below 1 mg in 200 cc of the extract, recourse was had to the sodium cobaltinitrite method as devised by Drushel (*7*, *8*) and modified by Morris (*14*). Further improvements have been introduced by Hibbard (*11*).

The determinations of such extremely low values of magnitude as were given in all the extracts with distilled water and with ammonium acetate after the first leachate were carried out by the colorimetric method of Schreiner and Failyer (*17*, *pp. 31–33*).

Difficulty has been experienced by some investigators with the platinic chloride method, especially on the extracts from ammonium salts, which are usually very high in calcium. When this method is used it is necessary for the analyst to assure himself of the complete removal of all traces of calcium before proceeding to the precipitation of the mixed chloroplatinates. This may be accomplished by treating the sirupy mixed chloroplatinates with 15 to 20 cc of a solution of acidified alcohol. The acidified alcohol is prepared by passing washed hydrochloric acid gas into commercial 95-percent alcohol until the solution is two to three times normal in strength. The usual procedure is then followed (*2*).

EXPERIMENTAL DATA

The total potash (K_2O) content of the three horizons of the original, check, NPK, NK, and PK soils was found to range between certain maximal and minimal values shown in the following tabulation:

Horizon	*Range of K_2O, percent*
Surface soils (0 to 7 inches)	3.919 to 4.007
Subsurface soils (7 to 21 inches)	4.197 to 4.205
Subsoils (21 to 53 inches)	4.405 to 4.410

The probable error of analyses may be gaged from 10 determinations made on the original surface soil before the experiment began. The results were 3.925 ± 0.005.

The results of the percolation experiments with water, M/2 ammonium acetate, and 0.2 N nitric acid are given in table 2.

TABLE 2.—*Potash (K_2O) in successive leachings with distilled water, M/2 ammonium acetate, and 0.2 N nitric acid, expressed in parts per million of dry soil and in grams*

DISTILLED WATER

Description of soil	Potash in successive leaching indicated										Total in first 3 leachings	Absolute amount in first 3 leachings	Total in all leachings	Absolute amount in all leachings
	1	2	3	4	5	6	7	8	9	10				
Soil before trees were planted:	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*Grams*	*P. p. m.*	*Grams*
0–7 inches	41.4	17.2	10.4	9.8	10.0	7.2	6.0	5.2	5.0	5.0	69.0	40.4	117.2	68.6
7–21 inches	27.9	12.0	9.8	10.0	8.5	6.4	6.4	6.0	5.5	5.5	49.7	58.6	98.0	115.6
21–53 inches	26.2	11.4	10.2	9.3	9.1	7.5	6.2	6.0	4.0	4.0	47.8	128.6	93.9	252.6
Check (sod):														
0–7 inches	44.7	15.2	12.7	10.1	9.4	10.0	9.9	9.7	9.8	9.8	72.6	42.5	141.3	82.7
7–21 inches	33.4	11.4	9.7	10.0	9.8	9.2	9.4	10.0	8.9	9.0	54.5	64.3	120.8	142.5
21–53 inches	26.9	10.7	10.3	9.0	9.1	8.0	9.4	9.7	9.0	9.0	47.9	128.8	111.1	298.8
Check (cultivation):														
0–7 inches	51.7	18.9	12.4	10.2	11.0	9.2	9.2	9.5	9.5	9.5	83.0	48.6	151.1	88.4
7–21 inches	37.5	14.2	10.7	9.0	9.5	9.5	8.0	9.0	9.0	9.0	62.4	73.6	125.4	147.9
21–53 inches	32.1	12.7	9.2	8.5	9.4	9.0	9.5	9.0	8.5	8.5	54.0	145.2	116.4	313.1
NPK (sod):														
0–7 inches	190.0	42.6	32.6	21.3	15.7	10.9	9.8	10.2	10.4	9.8	265.2	155.2	353.3	206.7
7–21 inches	77.2	27.4	15.6	10.8	11.1	9.2	9.2	9.0	9.5	9.0	120.2	141.7	188.0	221.7
21–53 inches	27.9	14.9	10.7	9.5	9.5	10.0	9.0	9.0	9.5	9.0	53.5	143.9	119.0	320.1
NPK (cultivation):														
0–7 inches	201.0	62.4	24.6	12.4	10.9	11.0	9.0	8.4	9.0	9.0	288.0	168.5	357.7	209.3
7–21 inches	82.6	29.4	14.2	10.0	9.0	9.0	8.5	8.5	8.5	8.5	126.2	148.8	188.2	221.9
21–53 inches	29.0	13.6	10.4	9.1	10.0	9.5	9.5	9.0	9.0	9.0	53.0	142.5	118.1	317.7
PK (sod):														
0–7 inches	177.4	35.4	[1] 27.9	.....	.....	.....	.....	.....	.....	.....	240.7	140.8	.....	.....
7–21 inches	80.4	25.2	[1] 12.9	.....	.....	.....	.....	.....	.....	.....	118.5	139.8	.....	.....
21–53 inches	25.7	16.2	[1] 9.4	.....	.....	.....	.....	.....	.....	.....	51.3	138.0	.....	.....
PK (cultivation):														
0–7 inches	188.2	33.7	[1] 25.4	.....	.....	.....	.....	.....	.....	.....	247.3	144.7	.....	.....
7–21 inches	90.4	25.6	[1] 12.2	.....	.....	.....	.....	.....	.....	.....	128.2	151.2	.....	.....
21–53 inches	28.2	12.9	[1] 7.2	.....	.....	.....	.....	.....	.....	.....	48.3	129.9	.....	.....

M/2 AMMONIUM ACETATE

Soil before trees were planted:														
0–7 inches	73.6	17.4	15.5	10.2	8.0	.....	.....	.....	.....	.....	106.5	62.3	124.7	73.0
7–21 inches	101.3	35.3	17.9	12.4	8.5	.....	.....	.....	.....	.....	154.5	182.2	175.4	206.8
21–53 inches	160.8	51.0	22.4	11.2	9.0	.....	.....	.....	.....	.....	234.2	629.9	254.4	684.3
Check (sod):														
0–7 inches	106.1	34.8	17.4	12.1	9.5	.....	.....	.....	.....	.....	158.3	92.6	179.9	105.3
7–21 inches	106.0	50.3	29.6	13.4	9.0	.....	.....	.....	.....	.....	185.9	219.2	208.3	245.6
21–53 inches	172.9	44.5	22.6	12.8	8.2	.....	.....	.....	.....	.....	240.0	645.5	261.0	702.0

Check (cultivation):														
0–7 inches	112.2	37.4	16.2	12.0	8.5						165.8	97.0	186.3	109.0
7–21 inches	116.0	52.4	28.7	14.2	9.0						197.1	232.4	220.3	259.8
21–53 inches	160.5	51.8	22.7	10.4	8.8						235.0	632.1	254.2	683.7
NPK (sod):														
0–7 inches	329.4	51.4	24.3	12.2	9.4						405.1	237.0	426.7	249.7
7–21 inches	204.8	51.2	24.9	11.8	9.0						280.9	331.3	301.7	355.8
21–53 inches	167.9	48.4	23.2	12.4	8.0						239.5	644.2	259.9	699.1
NPK (cultivation):														
0–7 inches	322.4	50.2	30.4	12.1	10.0						403.0	235.8	425.1	248.7
7–21 inches	219.2	53.7	27.8	11.0	10.2						300.7	354.6	321.9	379.6
21–53 inches	163.8	49.6	24.9	10.2	9.5						238.3	641.0	258.0	694.0
PK (sod):														
0–7 inches	342.4	60.4	[1] 28.2								431.0	252.2		
7–21 inches	200.8	47.2	[1] 22.8								270.8	319.4		
21–53 inches	168.2	50.4	[1] 26.4								245.0	659.0		
PK (cultivation):														
0–7 inches	319.6	58.2	[1] 39.4								417.2	244.1		
7–21 inches	210.4	52.4	[1] 29.2								292.0	344.4		
21–53 inches	162.9	45.6	[1] 23.4								231.9	623.8		

0.2 N NITRIC ACID

Soil before trees were planted:														
0–7 inches	196.3	104.7	72.4	53.9	37.4	29.6	25.4	23.2	22.4	20.6	373.4	218.5	585.9	342.8
7–21 inches	235.5	182.1	170.5	135.6	117.2	83.4	48.9	39.7	28.6	25.0	588.1	693.6	1,066.5	1,257.8
21–53 inches	347.8	213.1	187.9	168.6	149.2	92.1	70.6	49.4	37.4	30.6	748.8	2,014.1	1,346.7	3,622.3
Check (sod):														
0–7 inches	360.4	251.9	224.8	143.4	135.6	116.2	89.1	54.2	38.7	33.8	837.1	489.8	1,448.1	847.3
7–21 inches	392.6	203.4	156.9	143.3	120.6	112.4	91.1	42.6	39.7	35.4	752.9	887.9	1,338.0	1,577.9
21–53 inches	372.1	203.5	172.4	158.4	136.8	101.4	81.6	53.2	38.9	35.6	748.0	2,012.0	1,353.9	3,641.7
Check (cultivation):														
0–7 inches	347.3	262.6	219.8	144.6	130.8	119.7	88.6	52.4	39.6	38.4	829.7	485.5	1,443.8	844.8
7–21 inches	400.0	224.8	158.9	140.4	118.7	104.2	99.7	59.4	48.2	41.4	783.7	924.2	1,395.7	1,646.0
21–53 inches	370.4	200.6	158.4	150.2	144.5	102.4	81.4	71.8	52.6	40.9	729.4	1,961.9	1,373.2	3,693.6
NPK (sod):														
0–7 inches	830.0	244.2	235.9	135.0	96.9	88.4	77.5	61.0	42.1	38.4	1,310.1	766.6	1,849.4	1,082.1
7–21 inches	446.8	262.8	160.4	149.8	120.0	110.7	98.2	50.6	48.1	40.4	870.0	1,026.0	1,487.8	1,754 6
21–53 inches	382.2	208.4	178.4	172.8	137.8	112.4	91.4	63.8	58.9	42.6	769.0	2,068.4	1,448.7	3,896.7
NPK (cultivation):														
0–7 inches	817.4	239.4	[1] 220.6								1,277.4	747.4		
7–21 inches	459.2	260.4	[1] 179.4								899.0	1,060.2		
21–53 inches	370.6	208.0	[1] 190.4								769.0	2,068.4		
PK (sod):														
0–7 inches	848.8	147.2	135.6	116.2	116.2	100.6	93.0	77.2	60.4	40.1	1,131.6	662.1	1,735.3	1,015.4
7–21 inches	447.2	260.4	162.8	160.4	119.0	104.6	94.2	70.4	58.4	40.4	870.4	1,026.5	1,517.8	1,789.9
21–53 inches	383.8	208.4	178.6	166.4	138.2	109.4	92.8	70.4	42.8	31.4	770.8	2,073.3	1,422.2	3,825.4
PK (cultivation):														
0–7 inches	839.4	154.2	[1] 141.4								1,135.0	664.1		
7–21 inches	462.8	278.0	[1] 190.4								931.2	1,098.2		
21–53 inches	380.8	202.2	[1] 168.4								751.4	2,110.7		

[1] Percolation experiments were not carried further.

ANALYSIS AND DISCUSSION OF RESULTS

DOWNWARD MOVEMENT OF POTASSIUM

As Determined From Data for Total K_2O

The error involved in the determination of total potash, although relatively small, is absolutely large when multiplied by the weights of the soil in each horizon, viz, surface 1,290 pounds, subsurface 2,600 pounds, and subsoil 5,930 pounds. When the data for the total potash from each horizon were analyzed it was found that the error of analysis was too great to permit conclusions to be drawn with respect to the movement of potassium applied as fertilizer.

As Determined From Percolation Data

More accurate information should be obtained from extraction or percolation data. The amount of potassium which has moved from one horizon to another can be obtained by the difference between the amounts removed by the respective solvents from corresponding horizons of treated and check cylinders. The data so obtained are shown in table 3.

TABLE 3.—*Increase in the amounts of K_2O removed in 3 leachings by distilled water, M/2 ammonium acetate, and 0.2 N nitric acid from the NPK and PK cylinders above that of the corresponding horizons of the check cylinders*

Description of soil	Distilled water	M/2 ammonium acetate	0.2 N nitric acid
	Grams	*Grams*	*Grams*
NPK (sod):			
0–7 inches	113	144	277
7–21 inches	77	108	138
21–53 inches	6	−[1] 5	56
NPK (cultivation):			
0–7 inches	120	139	262
7–21 inches	75	127	136
21–53 inches	−[1] 1	9	53
PK (sod):			
0–7 inches	98	160	172
7–21 inches	76	96	139
21–53 inches	3	17	61
PK (cultivation):			
0–7 inches	98	147	180
7–21 inches	77	117	174
21–53 inches	−[1] 6	−[1] 8	60

[1] Decrease.

The progressive increases in these values (table 3) as one proceeds from the distilled water, M/2 ammonium acetate, and 0.2 N nitric acid extracts in the 7- to 21-inch and 21- to 53-inch horizons of the respective treatments indicate the condition with respect to solubility, in each solvent, of the applied potassium sulphate that has moved downward. The quantities of potassium that have so moved are expressed as a percentage of the residual applied potash in table 4. The residual amounts can be calculated from the known amounts of potash applied and that absorbed by the trees (*19*).

The subsurface data show that amounts ranging from 14.1 to 15.3 percent of the portion of the residual applied potash which has moved into the 7- to 21-inch layer are soluble in water; that amounts ranging from 17.9 to 26.4 percent are removed by M/2 ammonium acetate and 27.1 to 32.1 percent by 0.2 N nitric acid.

TABLE 4.—*Fraction of the applied potash found in the different leachates from the subsurface and subsoil horizons expressed as a percentage of the residual applied potash*

Horizon and treatment	Distilled H_2O	M/2 $NH_4.C_2H_3O_2$	0.2 N HNO_3
	Percent	*Percent*	*Percent*
Subsurface:			
NPK (sod)	15.1	21.0	27.1
NPK (cultivation)	15.3	26.4	28.3
PK (sod)	14.1	17.9	25.5
PK (cultivation)	14.2	21.6	32.1
Subsoil:			
NPK (sod)	1.2	----	11.0
NPK (cultivation)	----	1.9	11.0
PK (sod)	.5	3.2	11.4
PK (cultivation)	----	----	11.0

The subsoil data are more difficult to interpret. The percentages of the residual potash removed by distilled water and M/2 ammonium acetate probably lie within the limits of error of the experiment. The high results for the amounts removed by 0.2 N nitric acid therefore may not indicate a downward movement into this horizon, but may be a reflection of the increased solubility in this solvent of the native soil potash as the result of the effect of the far greater root systems in the treated cylinders.

CONDITION OF THE POTASSIUM

THE WATER-SOLUBLE POTASSIUM

Since distilled water will remove the salts that have not been adsorbed by the clay and humus complexes of the soil, leaching with this solvent in in effect similar to that of leaching with an electrolyte. Brönsted's investigations (*4*, *5*) indicate the character of solubility changes in the presence of electrolytes.

The concentration of potassium, which is relatively high in all of the first percolates with distilled water, rapidly falls in the second extracts, and the decrease is then more gradual as the electrolytes of the soil solution are removed. This gradual removal of electrolytes explains why the fraction of the so-called exchangeable potassium (p. — and table 5) removed by distilled water prior to the establishment of a constant concentration is so large.

TABLE 5.—*The quantities of so-called exchangeable potash removed in the first 3 leachings with distilled water*

Description of soil	Original soil	Check		NPK		PK	
		Sod	Cultivation	Sod	Cultivation	Sod	Cultivation
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Surface	64.8	45.9	50.1	65.5	71.5	55.8	59.3
Subsurface	32.2	29.3	31.7	42.8	42.0	43.8	43.9
Subsoil	20.4	19.9	23.0	22.3	22.2	20.9	20.8

The results show how impossible it is to determine the real quantities of water-soluble and exchangeable potassium. The water-soluble potassium must necessarily contain a certain amount of exchangeable potassium, because the leaching with water of these

soils containing relatively large amounts (p. 534) of applied potassium sulphate is in fact equivalent to leaching with a dilute solution of a neutral salt—in this case potassium sulphate—containing no ion in common with the salt used to determine the replaceable potash, viz, ammonium acetate. This phenomenon is further discussed in the next two sections from the point of view of the more general aspect of soil problems. Moreover, the ammonium acetate leachates will necessarily contain the water-soluble potash. It is, therefore, obvious that the "replaceable" potash cannot be definitely and exactly determined.

The very large percentages of the so-called exchangeable potash removed by water in these experiments is consequently explicable. Thus, from 46 to 50 percent of the so-called exchangeable potassium (p. 541) is removed by distilled water in the first three extracts from the surface soil of the check cylinders and from 65 to 71 percent from the NPK cylinders. Even in the surface layer of the original soil 65 percent of the exchangeable potassium is removed by water in the first three extracts. In the lowest horizons the fraction of the exchangeable potassium removed in the first three extracts is reduced to approximately 20 percent.

In the check and all treated cylinders the concentration of potassium in the eighth and succeeding leachates with distilled water is reduced to approximately 10 parts per million of the dry weight of the soil. In the original soil the concentration tends toward a relatively constant level of about 5 parts per million as leaching is continued. These differences in the constant level reached by the original soil and in the soils in the cylinders at the end of the experiment are significant. The stage reached by reduction to the constant level is indicative of the existence of a potassium combination of such low solubility that the amount going into solution at each extraction is independent of the mass present in the soil. The influences to which both the check and treated cylinders have been subjected, therefore, have resulted in an alteration in the character of this insoluble potassium combination in the direction of increasing solubility.

The question may now be asked, What significance do aqueous extracts of soils possess with respect to potassium content? Water extracts of soils obtained by displacement, 1:5 water extraction, and percolation methods have played a considerable role in investigations relating to the potassium-supplying power of soils. But, as already indicated (p. 539), the significance of the results needs to be reconsidered in the light of recent acquisitions to our knowledge of the behavior of the adsorbed materials in the presence of electrolytes of which, in soils, calcium is such a dominant factor. Although, as in these experiments, small amounts of potassium are dissolved for long periods of continuous leaching of a soil, this fact affords no indication that the difficultly soluble combinations of potassium would be removed by water in the absence of all traces of electrolytes. A low but effective concentration in electrolytes may be produced by several processes, such as (1) action of the micro-organisms (the soil was not sterile in these leaching experiments), (2) the effect of humic acids which can effect a repartition between the elements adsorbed by the humus-clay complexes and the solution phase in contact with it (*6*), and (3) the effect of the slow solubility of carbonates and of silicic

acids in water. Under natural field conditions the potassium fixed by the soil can enter into solution only as the electrolytes are replenished by the processes cited. It follows that the amount of potassium removed in aqueous extraction by any method whatsoever is indicative not only of the amount of "soluble" potassium but also of its temporary content of nitrates, chlorides, bicarbonates, sulphates, etc.

This effect of electrolytes on the solubility of adsorbed potassium is striking. The relationship quantitatively demonstrated by Wiegner and Müller (*23*) with permutite and with six soil types by Barbier (*3*) is, when the exchangeable hydrogen ions are relatively small compared with calcium

$$\frac{\frac{P}{C}}{1+\frac{P}{C}}=\frac{k}{T}\cdot\left(\frac{p}{c}\right)^{\frac{1}{n}}$$

where P and C are the concentrations of potassium and calcium in the adsorbent, p and c the concentration of these elements in the solution, k and n constants, and T the exchange capacity.

This formula shows that at equilibrium the ratio of potassium to calcium fixed in the adsorbing complex determines the ratio of potassium to calcium in solution. Accordingly, for one and the same concentratlon of potassium in the adsorbing complex at equilibrium, the concentration of potassium in the aqueous phase is never constant as the classical theory of adsorption holds. On the contrary, it is proportional to the fimal concentration of other cations, of which calcium, because of its relatively greater proportion to other cations in soils, plays the dominant role, and consequently, the concentration of potassium is proportional to the sum p plus c of potassium and calcium dissolved. This sum is equal to the quantity of anions present in the aqueous phase, i. e., to the total salt concentration of the solution. The concentration of the soil solution in potassium is then an increasing function of two factors (1) the ratio $\frac{P}{C}$ between the potassium and calcium "fixed" at equilibrium, which determines the ratio $\frac{p}{c}$ between potassium and calcium dissolved, and (2) the total salt concentration of the solution.

An appreciation of the significance of these facts will explain the difficulties encountered in all attempts to obtain threshold values for soil potassium; and, furthermore, will suggest the direction in which investigations on availability of soil potassium should proceed.

The So-Called Exchangeable Potassium

It follows from Wiegner's observations discussed above that the so-called exchangeable potassium is, subject to the limitations of definition of the term "exchangeable", a variable quantity determined by the conditions of the experiment. For the determination of this so-called exchangeable soil potassium, extraction with a solution of either sodium or ammonium acetate has certain advantages over other reagents (*16*). In the present experiments a half-molar solution of ammonium acetate was used. This strength is sufficiently high, especially for percolation methods. Even in one extraction

Barbier (*3*) found that a normal solution of ammonium chloride displaced the whole, and a one-third normal solution 98 percent, of the exchangeable potassium from clay soils.

The results given in table 2 show that under the conditions of continuous leaching adopted in these experiments three extractions with M/2 ammonium acetate were necessary before the concentration of potassium was reduced to that of the constant level reached by the water leachates in the fourth extraction. After the third percolation the amounts of potassium removed by M/2 ammonium acetate were nearly identical for all the soils of this experiment. The addition of the potassium sulphate fertilizer resulted in an increase of the so-called exchangeable potassium of the surface horizons of the treated cylinders approximately two and a half times above that of the check cylinders. The effect of the cover crops on the exchangeable potassium was also quite marked. This is indicated by increases of 50 and 75 percent in the surface and subsurface soils of the check cylinders, respectively, over the corresponding horizons of the original soil.

The residual quantities of potash shown in column 2 of table 6 can be calculated from the amounts of potassium sulphate applied together with that absorbed by the trees from the added potassium sulphate (*19*).

TABLE 6.—*Quantities of residual potash which have accumulated in the exchangeable form in the 0- to 21-inch layer*

Description of soil	Residual K_2O (x)	Excess of exchangeable K_2O of treated cylinders over that of check cylinders (y)	$\frac{y}{x} \times 100$
	Grams	*Grams*	*Percent*
NPK (sod)	508	253	50
NPK (cultivation)	481	266	55
PK (sod)	536	256	48
PK (cultivation)	541	264	49

The differences shown in table 6 between the exchangeable K_2O of the treated cylinders and the corresponding check cylinders of the 0- to 21-inch horizon indicate that about one-half of the potassium applied to the soils may be considered to be in a replaceable form. Actually the amounts must be somewhat lower (p. 541). The remainder, consequently, must have been transformed into a nonexchangeable form and the greater portion in the 0- to 7-inch horizon.

The possibility of the conversion of surface-adsorbed exchangeable bases into a nonexchangeable form and vice versa is suggested by the results of several investigators (*10, 12, 13, 15*). This experiment supplies additional and possibly less vulnerable evidence of the fact.

RELATIVE AMOUNTS OF RESIDUAL APPLIED POTASH REMOVED BY THE DIFFERENT SOLVENTS FROM THE 0- TO 21-INCH LAYER OF THE NPK CYLINDERS

The relative amounts of residual applied potash removed by the different solvents (total of all leachings in each case) can be determined in a manner similar to that used to determine the phosphoric acid removed by various solvents (*21*).

Table 7 gives the potash removed by the different solvents from the 0- to 21-inch horizon of the NPK cylinders expressed as a percentage of the residual applied potash. The amounts of potash dissolved by the unfertilized (check) soils by the respective solvents are subtracted from the quantities removed from the treated cylinders as shown in column 2, under the indicated method of calculation.

TABLE 7.—*Relative magnitudes of the potash removed by the different solvents from the 0- to 21-inch horizon of the NPK cylinders*

Solvent	Indicated method of calculation	Percentage of residual applied K_2O dissolved
	IN SOD	
	Grams	
Distilled H_2O	$\frac{428.4-225.2}{508}\times 100=$	40.0
M/2 $NH_4C_2H_3O_2$	$\frac{605.5-350.9}{508}\times 100=$	50.1
0.2 N HNO_3	$\frac{2,836.7-2,425.2}{508}\times 100=$	81.0
	IN CULTIVATION	
Distilled H_2O	$\frac{431.2-236.3}{481}\times 100=$	40.5
M/2 $NH_4C_2H_3O_2$	$\frac{628.3-368.8}{481}\times 100=$	53.9

Distilled water has removed 40 percent; M/2 ammonium acetate 50 to 54 percent, and 0.2 N nitric acid 81 percent, respectively, of the residual potash of the 0- to 21-inch layer. The relatively large percentages removed by distilled water as compared with that removed by M/2 ammonium acetate is related to the phenomenon already fully discussed (p. 541).

Eighty-one percent of the residual applied potash has been removed by 0.2 N nitric acid from the 0- to 21-inch horizon of the NPK cylinder in sod. These values give the relative condition of availability of the potash applied as the fertilizer potassium sulphate.

The potash removed by 0.2 N nitric acid has been designated by Fraps (*9*) as the "active" potash. It is surprising that the exchangeable potash of many soils reported by Fraps is from two to three times the amounts found by him to be soluble in 0.2 N nitric acid. In these cylinder experiments the potash dissolved from the corresponding horizons of the respective cylinders by 0.2 N nitric acid are from two to three times the values for the exchangeable potash even in the first two extractions alone. If the analyses are comparable with those here reported there can be no relation between the replaceable potash and that extracted by 0.2 N nitric acid from different soil types.

THEORETICAL FERTILIZER RATIO OF $N:P_2O_5:K_2O$ FOR APPLE TREES GROWING ON A HAGERSTOWN CLAY LOAM SOIL

It was pointed out in the introduction to this and the two preceding papers of this series (*20*, *21*) that the ratio in which nitrogen, phosphoric acid, and potash were absorbed by the NPK- and NP-

treated trees was 3.0:0.3:1.5, whereas the ratio in which these same fertilizer entities were applied was 3:8:4. The divergence between these ratios is great.

Certain contemporary German agronomists and horticulturists have sought to determine the fertilizer requirements of crops by modification of the quantities in which the principal fertilizer entities are found in the mature crop considered, by making allowance for the quantities of nitrogen leached and the phosphoric acid and potash fixed by the particular soil used.

The factors most frequently used by the German investigators have been for nitrogen, 0.33; for phosphoric acid, 0.50; and for potash, 0.25. The amount of each of these entities found in the mature crop is then multiplied by the proper factor and the resulting values are added to the amount of each entity in the crop.

Since the quantity of nitrogen in plants is roughly proportional to the supply, this is a rational procedure for determining the nitrogen requirement; but its applicability in determining the amounts of phosphoric acid and potash required is more limited because, owing to fixation, the amounts of these entities absorbed by the plant are not proportional to the supply. In a previous paper of this series (*21*) it was shown that the fixing capacity of any one soil type is a relative and not an absolute property; the amount retained is conditioned not only by the concentration of the phosphate or potassium ions but also by the concentration of other ions, such as hydrogen, silica, iron, aluminum, calcium, magnesium, etc. It is also obvious that the amounts fixed will depend upon the manner of application. By a large localized application of a phosphate or potash fertilizer, the soil in that immediate neighborhood may be completely saturated. It has also been shown in a preceding paper (*21*) that the iron and aluminum of the original soil was only 66 percent saturated with respect to phosphoric acid. At the end of the cylinder experiments, with a residual applied phosphoric acid and potash content of 1,040 and 508 g respectively, one-third and one-fifth, respectively, of this residual phosphoric acid and potash of the NPK cylinders under sod were transformed into very insoluble forms.

The proper values for nitrogen, phosphoric acid, and potash to be used in modifying the amounts absorbed by the trees would therefore become:

Nitrogen leached	$0.33 \times 152.3 = 50.2$ g
Phosphoric acid fixed	$0.33 \times 1{,}040 = 343.2$ g
Potash fixed	$0.20 \times 508 = 101.6$ g

Therefore, the theoretical quantities for application to the NPK cylinder under sod taken as an illustration would be:

Nitrogen	$152.3 + 50.2 = 202.5$ g
Phosphoric acid	$19.2 + 343.2 = 362.4$ g
Potash	$47.2 + 101.6 = 148.8$ g

where 152.3, 19.2, and 47.2 are the amounts of nitrogen, phosphoric acid, and potash absorbed by the NPK tree in sod from the added salts (*21*). The theoretical ratio thus derived is 202.5:362.4:148.8, or a 4.47:8.0:3.28 fertilizer. This compares with a ratio used in this experiment based on results in the experimental orchard of 3:8:4.

The reason, therefore, why the ratios in which the nitrogen, phosphoric acid, and potash absorbed by the trees differs so greatly from both the theoretical and "practical" ratios is now apparent.

SUMMARY

The distribution and condition of potash at the end of an experiment lasting 6½ years is given for three horizons of a Hagerstown clay loam soil contained in cylinders planted to apple trees and treated with different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate in different combinations.

Approximately one-third of the residual applied potassium has moved into the subsurface (7-to 21-inch) layer. There is no definite evidence of movement of applied potassium into the 21- to 53-inch layer.

Under the conditions of continuous leaching of these experiments the amounts of potash removed from the surface soils of the check and also of the treated cylinders by distilled water was more than half of that removed by M/2 ammonium acetate (the so-called exchangeable potash).

The necessity for the reconsideration of the significance of all water-soluble and so-called exchangeable potash data in soil investigations is discussed.

The system of green manuring adopted in this experiment has resulted in increasing the so-called exchangeable potassium of the 0- to 21-inch layer almost 50 percent.

Only about 50 percent of the residual applied potassium was found in a form soluble in M/2 ammonium acetate. The remainder has been converted into less soluble forms.

The relative quantities of the residual applied potash removed under conditions of continuous percolation from the 0- to 21-inch layer of the NPK-treated cylinder in sod by distilled water, M/2 ammonium acetate, and 0.2 N nitric acid were 40, 50, and 80 percent, respectively.

From the present and earlier reported experiments a theoretical fertilizer ratio for apple trees in a Hagerstown clay loam soil is derived. The theoretical ratio of $N:P_2O_5:K_2O$ is 4.47:8.0:3.28.

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FACTORS AFFECTING THE VITAMIN C CONTENT OF APPLES [1]

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INTRODUCTION

Marked variations in the vitamin C content of different varieties of apples have been observed. The values of 21 Massachusetts-grown varieties were reported by Smith and Fellers,[2] and the results of several other workers have been summarized by Batchelder.[3] Between different lots of apples of the same variety small differences in vitamin C concentration have been found which appeared to result from the kind of fertilizers used (Potter and Overholser).[4] Nelson and Mottern [5] report that the vitamin C content of oranges is low in fruit from trees that have been heavily sprayed with lead arsenate. It seemed possible that the adverse effect of the spraying might have resulted primarily from a lowering of the effective photosynthetic capacity of the leaf.

A change in the ratio of leaf area to fruit, effected by thinning of leaves to produce a low ratio and by thinning of undeveloped fruit to produce a high ratio, would afford a means of measuring directly the influence upon the vitamin C content of fruit of a change in its supply of the products of photosynthesis. Hence, in this investigation of the factors that affect the vitamin C content of apples, a study was made of the concentration of this vitamin in apples grown with a high ratio of leaf area to fruit as compared with apples grown with a low ratio of leaf area to fruit. A study was also made of size of individual apples as a factor in the measurement of the vitamin C content. Two varieties of apple, Delicious and Winesap, were used. Both varieties were grown with different ratios of leaf area to fruit in connection with the experimental work of the Division of Horticulture of the Washington Agricultural Experiment Station at Pullman. Each variety was grown under uniform conditions of fertilization, spraying, and other environmental factors, the sole variant being ratio of leaf area to fruit.

METHODS

Delicious apples of mature trees grown on large, unringed branches were adjusted the first week in July, in one case to an approximate leaf-fruit ratio of 60 leaves to each fruit and in another case to 20

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leaves to each fruit. Winesap apples were grown with similar leaf-fruit ratios, but upon ringed branches.

The apples were stored at 32° F. soon after harvesting, since results obtained the previous season with Delicious apples [6] had indicated that vitamin C is almost completely preserved at this temperature for the length of time covered by these experiments.

The method of vitamin C determination was similar to that hitherto employed in this laboratory, being a modification of the method of Sherman, La Mer, and Campbell.[7] In the first series of experiments, the apples were fed in radial sections.

RESULTS

VITAMIN C CONTENT AS AFFECTED BY RATIO OF LEAF AREA TO FRUIT

The results of the first series of experiments, with Delicious apples, are presented in table 1. Almost no difference was observed between the Delicious apples grown at the high leaf-fruit ratio and those grown at the low. The results obtained with 20 g of Delicious apple in each case corresponded closely with results obtained in earlier work by Batchelder [8] with check Delicious apples at this level. Guinea pigs receiving 23 g showed very slight scurvy, while those receiving 25 g in the previous study were completely protected. Apparently 25 g of Delicious apple represented very nearly the minimum protective dose according to the methods employed, with apples grown under the conditions prevailing in the station orchard. Different results reported from other laboratories may possibly be attributable to differences in size of apples or method of sampling, as later shown.

TABLE 1.—*Antiscorbutic potency of radial sections of Delicious and Winesap apples grown with high and low leaf-fruit ratios when fed to guinea pigs as the sole source of vitamin C*

Vitamin C supplement	Animals	Average daily gain or loss in weight	Average scurvy score
Delicious, high leaf-fruit ratio:	*Number*	*Grams*	
23 g	4	3.8	2.0
20 g	5	2.0	4.4
17 g	5	2.3	6.4
Delicious, low leaf-fruit ratio:			
23 g	6	1.8	2.2
20 g	6	1.7	5.5
17 g	5	1.7	6.2
Winesap, high leaf-fruit ratio: 8 g	8	1.6	9.9
Winesap, low leaf-fruit ratio: 8 g	8	1.8	4.9
Cabbage, 20 g [1]	11	3.1	.5
None [2]	2	−3.7	20.0

[1] Positive controls.
[2] Negative controls.

Contrary to the results obtained with Delicious apples, there was considerable difference between the Winesap apples grown at the two leaf-fruit ratios, those grown at the low ratio having a higher vitamin

[6] BATCHELDER, E. L. See footnote 3.
[7] SHERMAN, H. C., LA MER, V. K., and CAMPBELL, H. L. THE QUANTITATIVE DETERMINATION OF THE ANTISCORBUTIC VITAMIN (VITAMIN C). Jour. Amer. Chem. Soc. 44: 165–172, illus. 1922.
[8] BATCHELDER, E. L. See footnote 3.

C content than those grown at the high ratio. These apparently conflicting results with Delicious and Winesap apples may be explained on the basis of size, since the high- and low-ratio Winesaps showed the marked difference in size usually observed between apples grown under very different conditions; whereas the Delicious apples, presumably because the thinning was not completed sufficiently early and because the apples with the adjusted leaf-fruit ratios were not grown on ringed branches, were nearly the same in size and color, and, according to analyses, similar in chemical composition throughout.

VITAMIN C CONTENT AS AFFECTED BY SIZE OF APPLE

The question why the smaller Winesaps (grown at a low leaf-fruit ratio) were richer in vitamin C than the larger Winesaps (grown at a high leaf-fruit ratio) then presented itself. Theoretically, the size of the apple may cause a considerable difference in the scurvy-preventing action of radial sections of apples, or of apples which have been cored and ground before sampling, because there will, in both cases, be a higher proportion of skin in samples from small apples than in samples from large apples; and the skin is known to be much richer than the pulp in vitamin C.[9] Further study of the possibility that the difference between the vitamin C content of high and low leaf-fruit ratio Winesap apples might result from difference in size alone (the smaller apples showing weight for weight the higher vitamin C content) seemed desirable since otherwise it would apparently be necessary to conclude that the difference between the two types of Winesap was in inverse proportion to the nourishment received, and that the similarity of the two types of Delicious apple resulted from the fact that they were thinned too late and not grown on ringed branches, and hence were actually alike in their nutritive history.

A second comparison was therefore made to determine the effect of size on the vitamin C content of apples as ordinarily sampled. Check Winesap apples, the largest and smallest of the lot, were selected for comparison. Table 2 shows the results of feeding 8-g radial sections of large and small Winesap apples. This level was chosen as being probably slightly below the minimum protective dose and, therefore, capable of disclosing slight differences in vitamin C concentration. The average scurvy score shown by guinea pigs fed 8-g radial sections of large Winesaps (average weight 141 g) was somewhat higher than that of animals fed 8-g radial sections of small Winesaps (average weight 79 g), indicating that the large Winesaps contained, weight for weight, less vitamin C than the small Winesaps. Several guinea pigs refused to eat their apples and only four could be used in averaging the results. While a larger number of tests would have been desirable, the difference both in degree of scurvy and in rate of growth was so consistent that it seems certain that further tests would have strengthened the conclusion that the vitamin C supplied by an 8-g radial section of a large Winesap apple is measurably smaller than that supplied by a similar section of a small apple from the same tree.

[9] FELLERS, C. R., ISHAM, P. D., and SMITH G. G. VITAMIN C DISTRIBUTION IN BALDWIN AND M'INTOSH APPLES. Amer. Soc. Hort. Sci. Proc. (1932) 29: 93-97, illus. 1933.

TABLE 2.—*Antiscorbutic potency of large and of small Winesap apples: of sections containing peel and pulp from Winesap apples grown at high and at low leaf-fruit ratios, and of sections from Winesaps stored at 32° and at at 40° F. for 6 months, when fed to guinea pigs as the sole source of vitamin C*

Vitamin C supplement	Animals	Average daily weight gain	Average scurvy score
	Number	*Grams*	
LARGE AND SMALL WINESAP APPLES; 8-G PORTIONS			
Large Winesap	4	0.7	12
Small Winesap	4	1.6	9
SECTIONS CONTAINING PEEL PLUS ONE-FOURTH INCH OF UNDERLYING PULP FROM WINESAPS GROWN AT HIGH AND LOW LEAF-FRUIT RATIOS; 8-G PORTIONS			
Winesap, high leaf-fruit ratio	5	1.1	5
Winesap, low leaf-fruit ratio	6	2.1	4
RADIAL SECTIONS OF WINESAPS STORED AT DIFFERENT TEMPERATURES FOR 6 MONTHS; 10-G PORTIONS			
Winesaps stored at 32° F	5	1.5	6
Winesaps stored at 40° F	8	.3	11

VITAMIN C CONTENT AS AFFECTED BY RATIO OF SKIN TO PULP

A direct comparison of the vitamin C content of the skins of the two types of Winesaps presented difficulties in sampling because of the impracticability of completely removing adhering pulp. On the other hand, direct comparison of the pulps presented difficulties because of the large size of the sample required and the refusal of the guinea pigs to eat a sufficient quantity of the pulp. A method of sampling was therefore devised by which both difficulties were minimized. Table 2 gives the results of a comparison of high and low leaf-fruit ratio Winesaps sampled in such a way that the proportion of skin and pulp was the same for both the large and the small apples. The apples were so sampled that each section of skin had one-quarter inch of pulp attached. The curve of the outer skin was followed in cutting the pulp so that the resulting samples from large apples or from small apples consisted of pulp and skin in similar proportions. While variations in the amount of pulp included in each sample were not completely eliminated by this method of sampling, the variations represented a much smaller percentage of the total weight of the sample and caused a much smaller error in the apparent vitamin C content. The average weight of the high-ratio apples fed was 175 g, and that of the low-ratio apples 90 g. The average scurvy scores for animals fed 8 g of apple sampled as described above was five for the high-ratio and four for the low-ratio apples. These values indicated that, when ratio of skin to pulp was taken into account, the same concentration of vitamin C was present in the tissues of apples grown under widely different leaf-fruit ratios. For the reasons mentioned above, separate tests for pulp and skin were not attempted in connection with this study, but it may be inferred from the foregoing results that the concentration of vitamin C was similar for similar tissues of both large and small apples.

VITAMIN C CONTENT AS AFFECTED BY STORAGE OF APPLES AT 32° AND 40° F. FOR 7 MONTHS

Table 2 also gives the vitamin C value of Winesap apples held at 32° and 40°F. for 6 months. Apples of medium size (average weight 110 g.) were fed in 10-g radial sections. Apples stored at 32° produced only mild scurvy (average score 6), while those stored at 40° produced moderate scurvy (average score 11). Apparently the vitamin C content was better conserved at 32° than at 40°. Comparable results for these apples before storage were not available so that definite conclusions cannot be drawn as to the loss, if any, of vitamin C during 6 months' storage at 32°. Previous work, summarized by Batchelder,[10] indicated that 10 g of Winesap apples completely protected guinea pigs from scurvy. But possible differences in the size of the apples, fertilization of the trees, and other factors make direct comparisons of the vitamin C value of the stored apples reported here with that found in previously reported experiments on freshly harvested Winesaps, inadvisable.

SUMMARY AND CONCLUSIONS

The vitamin C content of apples was studied with respect to the effect of (1) the ratio of leaf area to fruit; (2) the size of fruit as it affected the ratio of skin to pulp, by various methods of sampling; and (3) storage at 32° and 40° F. for 6 months. It was concluded that the ratio of leaf area to fruit affected the vitamin C content of apples only indirectly, i. e., as it affected the size of the fruit produced; that the size of the fruit was an important consideration under common methods of sampling because the ratio of skin to pulp was higher in small apples than in large apples, and the skin contained a higher concentration of vitamin C than the pulp; that storage at 40° resulted in greater loss of vitamin C than did storage at 32°; and that the vitamin C content of Delicious apples in the present study corresponded closely with values hitherto reported from this laboratory.

[10] BATCHELDER, E. L. See footnote 3.

THE DETERMINATION OF THE APPARENT DIGESTIBILITY OF GREEN AND CURED GRASS BY MODIFIED PROCEDURES [1]

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INTRODUCTION

In 1926 Bergeim (*1*)[2] presented a simplified method for determining food digestibility and utilization that has certain advantages over the standard method. His method consists in adding iron oxide to the food and determining the ratio of the amount of a given food substance to the amount of iron in the feed and feces. The percentage utilization is calculated from these ratios. The ratio of a nutrient to iron in the feces when divided by the ratio of the nutrient to iron in the feed and multiplied by 100 gives the percentage of the nutrient not utilized. The percentage subtracted from 100 gives the percentage digested.

The following formula illustrates the method of computation:

$$100-\left(\frac{\text{Percent nutrient in feces}}{\text{Percent iron in feces}}\times\frac{\text{Percent iron in feed}}{\text{Percent nutrient in feed}}\right)100=\text{apparent digestibility.}$$

Bergeim proposed the use of iron oxide as a key substance because it is not appreciably absorbed from the intestines and is excreted practically in its entirety in the feces.

Gallup (*2*) compared the method proposed by Bergeim with the usual method of determining the digestibility of protein, which consists in measuring the amount of protein consumed and the amount excreted in the feces for a given length of time. This comparison was made with rats fed cottonseed-meal products. The apparent digestibility obtained by the Bergeim method was found to be slightly lower than that obtained by the standard method. Gallup attributed the lower figures to the loss of iron as it passed through the alimentary tract.

Investigations by Heller, Breedlove, and Likely (*5*) indicate that the normal iron content of a ration is a more accurate key substance than added iron oxide because it insures a more uniform intake and a more evenly distributed iron excretion in the feces. They concluded from investigations with rats that results obtained by the Bergeim method are comparable to those obtained by the old method. Comparisons of the apparent digestibility of the various feed constitutents determined by the two methods show fluctuations in results for the iron method both above and below the results obtained by the standard method.

[1] Received for publication Apr. 15, 1936; issued November 1936. Scientific Paper No. 341, College of Agriculture and Experiment Station, State College of Washington.

[2] Reference is made by number (italic) to Literature Cited, p. 556.

Gallup (*3*), in determining the digestibility of protein with rats, used silica as a key substance in place of iron oxide. His experiments with silica showed a smaller variation from the usual method than when iron oxide was used.

Gallup and Kuhlman (*4*) conducted an experiment with yearling Jersey heifers to determine by modified methods the digestibility of protein in cottonseed meal. They found that the coefficients of digestibility for the protein in the rations fed agreed very closely with those obtained in previous experiments by the ordinary methods. These investigators found that naturally occurring silica in the feed "served as a better index of the digestibility of the other substances than did the iron which had been added for that purpose."

METHODS

Additional work was necessary to determine the applicability of this modified method to nutritional work with larger animals. In conjunction with a number of digestion experiments, iron and silica determinations were made and apparent digestibility was calculated by the modified procedure and compared with results obtained by the standard method. Naturally occurring silica and naturally occurring iron were used as the key substances. The first digestion experiments conducted were with three 2-year-old Holstein heifers. In the first experiment these heifers were fed solely on artificially dried pasture grass, and in the second, solely on dried apple pomace. These experiments were conducted in the usual manner as described in previous publications (*6*, *8*).

In addition, silica and iron determinations were made on the feed and on the feces. Under the conditions of these experiments, it was found that silica was unreliable as a reference substance because of the impracticability of preventing contamination of the feed and feces. The wind blows frequently and vigorously in the section in which the experiments were conducted and considerable dust gets into both feed and feces, thus making the silica content an unreliable criterion. Moreover, considerable dirt may be incorporated in the feed at the time of harvest. Methods for the determination of the small amounts of naturally occurring iron also were inadequate.

In a digestion experiment with pea straw, iron and silica determinations were made. The results of this experiment confirmed the findings of the previous trials both with regard to the contamination by dust and also the lack of accuracy of determinations of small amounts of iron by the method used.

Experiments were undertaken to determine the effect of the temperature of drying on the digestibility of artificially dried pasture grass. Three wether sheep were used and six experiments were conducted. The methods of experimentation and the results obtained by the standard method have been reported in a previous paper (*7*). Improvement in methods of determining small amounts of iron in the feed and feces through the use of a photoelectric scopeometer made possible accurate and consistent iron determinations in this series of experiments. Three sheep were used in each of five experiments and two in the other and digestibilities were calculated for dry matter, crude protein, crude fiber, ether extract, and nitrogen-free extract for each sheep in each experiment.

RESULTS

A comparison of the apparent digestibility of nutrients in the feeds as determined by the modified and the standard method is given in table 1. The apparent digestibility calculated by the modified method varied both above and below that obtained by the standard method. The average percentage variation in digestibility of the modified method from the standard is given, together with the ratio of the difference to its probable error. This ratio indicates the significance of the variation. The variation among the individual animals in each digestion experiment for each nutrient is not materially greater by the modified than by the standard method.

TABLE 1.—*Comparison of apparent digestibility of nutrients in green, sun-cured, and artificially dried grass when fed to sheep, as determined by the standard and the modified (iron) method*

Treatment of grass fed	Sheep no.	Crude protein			Crude fiber			Ether extract			Nitrogen-free extract		
		Standard method	Modified method	Difference	Standard method	Modified method	Difference	Standard method	Modified method	Difference	Standard method	Modified method	Difference
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Green	1	74.3	72.2	−2.1	77.6	75.8	−1.8	73.4	71.2	−2.2	77.9	76.0	−1.9
	2	70.1	71.1	+1.0	71.4	72.3	+.9	63.3	64.5	+1.2	74.3	75.2	+.9
	3	72.7	71.2	−1.5	75.2	73.7	−1.5	75.8	74.4	−1.4	76.8	75.5	−1.3
Sun-cured	1	78.3	82.3	+4.0	84.5	87.4	+2.9	63.9	70.6	+6.7	82.6	85.8	+3.2
	2	78.5	84.1	+5.6	83.1	87.5	+4.4	68.5	76.7	+8.2	82.1	86.8	+4.7
Artificially dried at 250° F	1	75.4	80.4	+5.0	82.6	86.1	+3.5	73.2	66.5	−6.7	83.5	86.9	+3.4
	2	77.5	84.2	+6.7	82.1	87.5	+5.4	86.0	80.1	−5.9	83.0	88.1	+5.1
	4	75.3	83.2	+7.9	82.6	88.2	+5.6	79.3	69.4	−9.9	83.2	88.6	+5.4
Artificially dried at 300° F	1	75.4	69.2	−6.2	82.8	78.3	−4.5	67.4	74.0	+6.6	82.2	77.7	−4.5
	2	76.2	72.3	−3.9	82.4	79.4	−3.0	73.0	76.9	+3.9	82.2	79.3	−2.9
	4	74.8	74.0	−.8	80.6	79.7	−.9	81.2	81.9	−.7	81.6	81.5	−.1
Artificially dried at 350° F	1	76.0	76.1	+.1	85.3	85.0	−.3	70.0	70.5	+.5	85.2	85.0	−.2
	2	75.4	78.3	+2.9	84.0	85.8	+1.8	81.2	78.7	−2.5	82.7	84.8	+2.1
	4	74.8	77.8	+3.0	84.2	86.0	+1.8	82.4	80.5	−1.9	83.5	84.9	+1.4
Artificially dried at 400° F	1	58.1	64.3	+6.2	81.3	84.1	+2.8	79.6	76.0	−3.6	76.8	80.2	+3.4
	2	59.6	66.7	+7.1	80.2	83.7	+3.5	86.4	83.5	−2.9	75.7	79.9	+4.2
	4	59.7	66.5	+6.8	81.1	84.3	+3.2	87.9	85.4	−2.5	76.4	80.4	+4.0
Average percent variation of modified from standard method	----	5.9 ±0.6			3.4 ±0.3			5.4 ±.7			3.5 ±0.3		
Standard deviation	----	3.72± .43			1.82± .21			3.94±.46			2.02± .23		
Ratio of average percent variation to its probable error	----	9.7			11.4			7.7			11.7		

Since the modified method of calculating apparent digestibility is dependent upon the passage of the iron through the digestive tract without absorption therefrom or additions thereto, the iron balance should provide a measure of the accuracy of the procedure. As shown in table 2, this balance varied from a retention for the 14-day period of over 20 percent of the iron ingested to an iron content of the feces of over 40 percent in excess of that ingested. In 6 cases less iron was excreted than ingested and in 11 cases more iron was excreted than ingested. These results indicate that in the case of ruminants there is not a uniform passage of the naturally occurring iron in the feed through the digestive system.

TABLE 2.—*Iron-balance data for sheep when fed green, sun-cured, and artificially dried grass*

Treatment of grass	Sheep no.	Iron ingested	Iron voided	Iron balance	Iron balance as retention of ingested iron
		Grams	*Grams*	*Grams*	*Percent*
Green	1	20.6	19.0	+1.6	7.8
	2	21.7	22.5	−.8	3.7
	3	17.1	16.1	+1.0	5.8
Sun-cured	1	18.0	22.0	−4.0	22.2
	2	18.0	24.3	−6.3	35.0
Artificially dried at 250° F	1	11.6	14.5	−2.9	25.0
	2	11.6	16.5	−4.9	42.2
	4	11.6	17.1	−5.5	47.4
Artificially dried at 300° F	1	17.9	14.3	+3.6	20.1
	2	17.9	15.4	+2.5	14.0
	4	17.9	17.2	+.7	3.9
Artificially dried at 350° F	1	11.7	11.5	+.2	1.7
	2	12.8	14.5	−1.7	13.3
	4	12.8	14.5	−1.7	13.3
Artificially dried at 400° F	1	13.5	15.9	−2.4	17.8
	2	14.8	18.0	−3.2	21.6
	4	14.8	17.8	−3.0	20.3

SUMMARY

A modified method of determining apparent digestibility was compared with the standard method in six digestion experiments with 17 sheep. The results indicate that the variations of the modified from the standard method were statistically significant.

The variations obtained in iron balances and apparent digestibility by the modified method in these experiments indicate that the use of this method is not justified in the case of ruminants.

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